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HYPOPHYSECTOMY DURING PREGNANCY IN A PATIENT WITH CANCER OF THE BREAST: CASE REPORT WITH HORMONE STUDIES*

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ABSTRACT

Primary hypophysectomy and radiation therapy to the breast were decided upon as treatment for a woman with metastatic mammary cancer, first seen when 26 weeks pregnant. Replacement therapy for the duration of pregnancy (except for four days in week 32) consisted of 75 mg. of cortisone and 90 mg. of thyroid daily. Delivery was induced at 35 weeks because of premature labor and hormonal evidence of placental deficiency. Labor progressed normally without oxytocic stimulation. A normal 5-pound infant was delivered. There was no mammary secretion post partum. Pitressin, which had been required for control of diabetes insipidus after hypophysectomy, was no longer needed. Twelve days post partum, pneumonitis developed and terminated in death four weeks later. Autopsy revealed an organizing pneumonitis of both lungs involving all lobes. The only residual cancer consisted of islets of poorly differentiated cells

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in the fibrous stroma of one small axillary node. There was no residual pituitary tissue.

Hypophysectomy *per se* did not affect placental function as determined by the urinary excretion of chorionic gonadotropin, estrogens and pregnanediol. Aldosterone excretion, serum thyroxine-binding protein concentration and the electrophoretic pattern of serum were likewise unaffected. At 31 weeks there was no clinical evidence that replacement therapy was inadequate, but studies of serum PBI levels, of corticosteroid metabolism, and of glucose and insulin tolerance indicated a deficiency. When cortisone was stopped for two days and thyroid for four days, clinical signs of acute adrenal insufficiency appeared and were accompanied by chemical evidence of complete lack of endogenous adrenocortical hormone. This episode was followed by urinary findings indicative of placental failure and then by premature uterine contractions. Also observed was a drop in the level of serum total proteins, with lowered albumin and elevated gamma globulin ratios. There was continued chemical evidence of adrenal insufficiency. In the early postpartum period, during cortisone and thyroid therapy, the signs of adrenal insufficiency disappeared, glucose tolerance became normal, and an insulin tolerance test elicited the expected reaction.

CARCINOMA of the breast occurring in the pregnant woman carries a worse prognosis than in the non-pregnant woman (1) because of the exacerbation of the disease so commonly seen following delivery. Interruption of pregnancy after the first trimester does not improve the prognosis (2). Although slightly improved results have been reported from measures aimed at inhibiting the postpartum release of pituitary hormones, the pregnant patient with mammary cancer still presents a serious problem in which pituitary factors appear to be involved. Hypophysectomy has been used to control the disease and achieve some palliation in non-pregnant women. Furthermore, it has been demonstrated in primates that the pituitary gland is not necessary for the maintenance of pregnancy (3).

For these reasons it was decided to perform a primary hypophysectomy on the patient reported here, whose metastatic mammary cancer was first diagnosed during the second trimester of pregnancy. The local disease was treated with radiation therapy and the pregnancy was allowed to continue. She died five weeks after delivery, of intercurrent disease unrelated to the tumor, which remained well controlled.

Because the patient presented an unusual endocrine state, a concerted effort was made by several laboratories to study the endocrine changes involved. It is the purpose of this paper to report this case and describe the results of study.

I. CLINICAL

D.D. (P.B.B.H. #3K964), a 37-year-old gravida 4, para 3, white female was first admitted to the Peter Bent Brigham Hospital on December 26, 1956. At the time of admission the patient was 23 weeks pregnant, and the expected date of delivery was April 18, 1957. In September, 1956, when 8 weeks pregnant, she had been hospitalized

for an episode of vaginal bleeding diagnosed as threatened abortion. Seven months prior to admission, masses had been noted in the left axilla and breast. In late November 1956, a biopsy of these masses had revealed adenocarcinoma of the breast, with metastasis to a lymph node.

Physical examination on admission revealed a well nourished woman in good general condition. There was a mass in the upper outer quadrant of the left breast and a nodule in the left axilla. Both areas showed a recent incision. No abnormal signs were detected in the chest. The liver was not palpable, and the uterine enlargement was consistent with the twenty-third week of gestation.

Hypophysectomy was performed on January 7, 1957, with minimal trauma to the hypophyseal stalk.

The patient's postoperative course was initially uneventful. On the seventeenth day she had several grand-mal convulsions. These did not recur. Diabetes insipidus developed—evident on the first postoperative day. In keeping with current practice in treating this complication, vasopressin (Pitressin) was not administered until the seventh day. Urinary excretion was matched with oral intake of fluid.

Radiation to the left breast, axilla and internal mammary areas was begun on the seventh postoperative day and continued daily thereafter. The patient was discharged on February 9, 1957, her medication being 75 mg. of cortisone and 90 mg. of desiccated thyroid daily. Antidiuretic hormone therapy at this time consisted of 3.3 units of Pitressin tannate in oil every second day.

Seven days after discharge, the patient was admitted to the Boston Lying-in Hospital for further studies. Administration of thyroid was discontinued on the second hospital day, and of cortisone one day later. After two days without cortisone, signs of adrenal insufficiency developed, which gradually subsided after re-institution of cortisone therapy. Thyroid was re-started one day later. With the exception of the obstinate diabetes insipidus, her general clinical condition seemed satisfactory.

Because of the development of severe epithelitis over the left breast, radiation therapy was discontinued on February 20, 1957. It was resumed after the skin reaction had subsided, twelve days later. Subsequently, treatment was given daily until parturition.

After ten days of intermittent premature uterine contractions, and with hormonal evidence of failing placental function, labor was induced by rupture of the membranes on March 14, 1957. The labor was characterized by strong, unusually long contractions lasting 90/120 seconds. After twelve hours of labor, the patient delivered a female infant (5 pounds 1 ounce) uneventfully and without anesthesia. During labor she received 100 mg. of hydrocortisone by slow intravenous infusion.

Cortisone dosage, which had been elevated to 200 mg. for the first day following delivery, was then reduced to a maintenance level of 75 mg. per day. Thyroid therapy was continued at 90 mg. per day. The polyuria gradually decreased and administration of Pitressin was no longer required to maintain normal urine volumes, after the ninth postpartum day.[1] Radiation therapy was re-instituted on the fourth postpartum day, for seven days. The direct total tumor dosage was 2688 r; internal mammary irradiation, 2430 r; to the left axilla 3700 r; and in the left supraclavicular area, 4000 r.

Colostrum secretion and lactation, which had been copious after previous pregnancies, did not occur. Both breasts remained soft and were not tender to palpation throughout the entire postpartum period.

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[1] Observations on the changing Pitressin requirement following delivery are being reported separately.

On the eleventh postpartum day ominous signs appeared which were to lead to a fatal outcome, seemingly unrelated either to the hypophysectomy or to the pregnancy. Spiking temperature elevations, chills and a slight cough developed. Three days later, after the pulmonary symptoms had become progressively worse, bronchovesicular breath sounds were heard over the left upper lobe posteriorly, and a radiograph of the chest revealed infiltration of the left upper lobe. Cortisone dosage was increased to an average of 150 mg. per day, and antibiotic therapy was started.

The patient's subsequent course was one of progressive deterioration. On April 5, 1957, bronchoscopy was performed for left upper lobe atelectasis with mediastinal shift. A culture of the bronchoscopic washings and repeated cultures of the sputum failed to show any bacterial etiology for the pneumonitis. Blood cultures showed no growth. Despite administration of oxygen, humidification, and increased steroid and antibiotic therapy, her condition was marked by progressive failure of oxygenation. Studies four days prior to death revealed alkalosis, hypocapnia, and decreased oxygen saturation of arterial blood in the presence of a volumetrically adequate ventilation. This was felt to indicate alveolar capillary obstruction. The patient died on April 21, 1957. Death was characterized by inadequate oxygenation due to a defect in alveolar diffusion.

At autopsy the lungs were found to be practically airless, and the entire parenchyma of both lungs was involved by organizing pneumonia. Microscopic examination revealed marked proliferation of fibrous tissue and extensive obliteration of alveoli with few remaining alveolar spaces.

The adrenals, ovaries, pancreas and thyroid were atrophic, and no pituitary tissue was found in the sella turcica. Microscopic section of both breasts revealed involution of a pre-lactating breast and atrophy of breast tissue.

The only evidence of residual carcinoma was a small mass in a left axillary gland. In this there were islands of poorly differentiated cells showing mitotic activity in dense fibrous stroma. This clinical picture and these pathologic findings have not been seen in other patients under our care who have been treated by long-term cortisone therapy or by hypophysectomy. Neither have they been observed at other clinics where similar methods are used for treatment of carcinoma of the breast.[2]

The infant appeared pale at birth. Subsequently, progressive anemia developed, similar to that which had occurred in a previous child. Despite this, the infant gained weight at a satisfactory rate. Correction of the anemia was accomplished by several transfusions of packed red cells totalling 95 ml. The infant was discharged at the age of 7 weeks in excellent clinical condition.

II. INTRODUCTION TO SPECIAL STUDIES

Urinary chorionic gonadotropin was measured before and after hypophysectomy and up to the time of delivery. Estrogen and progesterone metabolites were followed from the thirty-first week through labor. Aldosterone excretion was measured before and after hypophysectomy and in the post partum period. The uptake of radioactive iodine (I^{131}) by the thyroid gland and the level of serum protein-bound iodine were measured before and after hypophysectomy. Random samples of serum were analyzed for protein-bound iodine, butanol-extractable iodine and thyroxine-binding protein both before and after hypophysectomy and also in conjunction with the corticoid studies.

[2] Personal communications from Drs. H. Atkins, R. Luft and O. H. Pearson.

A primary purpose of this study had been to determine whether or not adrenocorticotropin (ACTH) or corticoid-like hormones from the placenta (4, 5) might supplement or even replace the need for administration of cortisone to this patient during pregnancy. It was for this reason that replacement therapy was withdrawn at 32 weeks. The unexpected and alarming reaction after less than forty-eight hours without cortisone made it inadvisable to proceed further with this line of investigation. The serum level of 17-hydroxycorticosteroids and the urinary excretion of 17-ketosteroids and corticoids were followed from the thirty-first week through the puerperium. Cortisol infusions were given, with and without cortisone therapy, as well as post partum. Concurrent measurements of urinary 17-ketosteroids and 17-hydroxycorticosteroids were made.

Glucose and insulin tolerance tests were performed at 31 weeks and 5 days post partum. Serum electrophoretic patterns were determined repeatedly from the thirty-first week to delivery, and were also determined on umbilical cord serum.

III. METHODS

Collection of urine. All specimens were kept on ice during the period of collection and were either extracted on the day of completion (sodium pregnanediol glucuronide complex (NaPG) and estrogens) or deep-frozen and stored until extractions could be performed. The creatinine content of the urines was determined as a check on the accuracy of collection.

Chorionic gonadotropin (C.G.). Prior to the thirty-first week of pregnancy, determinations of urinary chorionic gonadotropin were made using male toads (*Bufo marinus*). These assays were carried out in the laboratory of Dr. Somers Sturgis. Thereafter serial dilutions of urine were tested on frogs (*Rana pipiens*) and check assays were performed using the rat test as described by Smith and Smith (6). Kaolin concentrates were used when the urine was too dilute. Comparisons with standards were made, and results reported in international units per twenty-four hours.

Sodium pregnanediol glucuronide complex (NaPG). The sodium "pregnanediol" glucuronide complex (NaPG) was gravimetrically measured by the method of Venning (7), results being calculated as pregnanediol. Each sample of crystalline NaPG was then hydrolyzed with beef liver β-glucuronidase and the neutral fraction prepared and colorimetrically analyzed for pregnanediol and 20-ketosteroids (8). Periodic-acid oxidation for 17-ketogenic steroids was also performed (8) to correct pregnanediol color assays for the presence of pregnane-3α, 17α, 20α-triol.

Estrogens (biologic and chemical assay). Urinary estrogens were measured in the phenolic fractions of urine subjected to glucuronidase hydrolysis (9). The results of chemical assay by the method of Brown (10) were compared with those of biologic assay (11). For bio-assay the strongly phenolic (estriol) and the weakly phenolic (estradiol and estrone) fractions were assayed prior to methylation, after the estrone and estradiol fractions had been separated by the micro-Girard procedure of Pincus and Pearlman (12). In current studies on estrogens in the urine of women ((9) and unpublished data[3]),

[3] Smith, O. W., and Smith, G. V.: Estrogens in the urine of women, with especial reference to hydrolytic procedures (Part III), *Acta endocrinol.* (in press).

TABLE 1. URINARY ESTROGENS

Bio-assay versus chemical assay before, during, and after interruption of replacement hormone therapy

Week of pregnancy	Date of urine sample	Estriol fraction			Estrone fraction			Estradiol fraction		
		Chemical assay		Biol.	Chemical assay		Biol.	Chemical assay		Biol.
		(μg./24 hrs. ×2 = RU /24 hrs.)		(RU/24 hrs.)	(μg./24 hrs. ×1.6 = RU/24 hrs.)		RU/24 hrs.)	(μg./24 hrs. ×20 = RU/24 hrs.)		RU/24 hrs.)
31	Feb. 13	5,150	10,300	20,000	62	100	400	38	760	800
31	Feb. 16	3,700	7,400	21,500	50	80	400	33	660	800
31	Feb. 17	4,750	9,500	20,000	70	122	400	42	840	1,070
	Thyroid discontinued Feb. 17 P.M.				Cortisone discontinued Feb. 18 P.M.					
32	Feb. 21	4,150	8,300	15,700	53	85	156	35	700	620
	Thyroid resumed Feb. 23 A.M.				Cortisone resumed Feb. 22 A.M.					
33	Feb. 27	3,550	7,100	9,100	46	74	167	31	620	1,200
34	Mar. 7	3,900	7,800	15,000	48	77	200	27	540	800
35	Mar. 14	5,150	10,300	24,500	94	150	370	65	1,300	1,480
Labor*	Mar. 15	5,050	10,100	16,800	77	123	170	50	1,000	1,060

* Induced.

it has been found that considerably more estrogenic material is measured by bio-assay than can be accounted for by the estriol, estrone and estradiol measured chemically by the Brown procedure. For this reason bio-assay results cannot be converted to micrograms, and all results are expressed in rat units per 24-hour volume. The conversion of micrograms to rat units is based on concurrent standardization bio-assays of estradiol, estrone and estriol (Table 1).

Plasma and urinary corticoids. Plasma corticoid values were determined in duplicate by the Nelson-Samuels method (13), using crystalline cortisol[4] as standard. Cortisol infusion consisted of the intravenous injection of 100 mg. of 17-hydroxycorticosterone over a period of five minutes. Plasma 17-hydroxycorticosteroid levels were measured before, and at one, two, four and six hours after injection, with the patient in a fasting state.

Urinary 17-hydroxycorticosteroids were estimated by the Glenn-Nelson method (14). Measurements of total urinary corticoids were made on aliquots of 24-hour urine collections and on 4-hour and 6-hour collections on the days cortisol was infused. The 17-ketosteroids were determined on the same urine, using the method of Drekter *et al.* (15).

Aldosterone. Aldosterone was measured during a period of measured sodium intake, employing a modification of Neher and Wettstein's method (16). The 24-hour samples of urine were subjected to hydrolysis at pH 1 for forty-eight hours, extracted with chloroform, chromatographed on a Florisil column and fractionated on paper using the methods of Zaffaroni (17) and of Bush (18). The final estimation was by the blue tetrazolium reaction (19).

Glucose tolerance and insulin tolerance tests. Glucose tolerance was estimated in a fasting state after the ingestion of 100 grams of glucose orally, after the patient had been prepared by ingestion 300 grams (total) of carbohydrate in the three days preceding the

[4] Cortisol refers to Kendall's compound F, or hydrocortisone, or 17-hydroxycorticosterone.

test. Estimations of blood sugar were made by the micro method of Folin *et al.* (20) on samples taken fasting, one, two and three hours after ingestion of glucose.

Insulin tolerance was determined by estimations of blood sugar on samples taken fasting and at ten, twenty, thirty, forty-five, sixty and one-hundred and twenty minutes after the injection of 0.1 unit of crystalline zinc insulin per kilogram of body weight.

Electrophoresis. Maternal and fetal serum protein patterns were determined by paper electrophoresis, employing a modification of Swahn's method (21), in which papers were run horizontally in a barbital buffer pH 8.6 at 4°C. using 4 to 5 volts per centimeter across the suspended paper. The papers were stained with amido black and analyzed with a densitometer.

Thyroid function tests. The uptake of radioactive iodine by the thyroid was measured twenty-four hours following the administration of a tracer dose of I^{131}. Serum protein-bound iodine levels were determined by a modification of the method of Zak *et al.* (22). Serum butanol-extractable iodine determinations were performed in the laboratory of Dr. E. B. Man at Yale University, New Haven. Thyroxine-binding protein studies were made by Dr. Norbert Freinkel and Dr. Thomas Dowling at the Thorndike Memorial Laboratories, Boston City Hospital.

IV. RESULTS AND DISCUSSION

General results of the hormonal investigations before and after hypophysectomy, during labor, during delivery, and in the postpartum period are graphically presented in Figure 1. Each aspect is separately reported and discussed here by the investigators concerned.

Aldosterone

In normal subjects with a sodium intake of 4 to 8 Gm. per day, the urinary excretion of aldosterone is 5.0 ± 3.0 μg. per twenty-four hours. The increased output of aldosterone reported in pregnancy (23, 24) is confirmed by the present patient's mean preoperative excretion of 14 μg. per twenty-four hours. After hypophysectomy the values were higher, and on the fifth postoperative day rose to 106 μg. per twenty-four hours. During Pitressin therapy they returned to the preoperative level. Three factors may have contributed to this rise. In the first place, increased excretion of aldosterone has been reported following surgery (25). The findings presented here show that this can occur following hypophysectomy, thus favoring the concept that the secretion of aldosterone is independent of pituitary control (26). Secondly, it is pertinent that the diabetes insipidus which developed following hypophysectomy was deliberately not controlled for six days; after the patient received Pitressin her aldosterone output decreased. It is known that in established cases of diabetes insipidus the patients excrete increased amounts of aldosterone when not receiving Pitressin (27) and while presumably suffering a reduction in extracellular fluid volume. The third factor contributing to the increased postoperative output of aldosterone may have been the lower intake of sodium during the five days after hy-

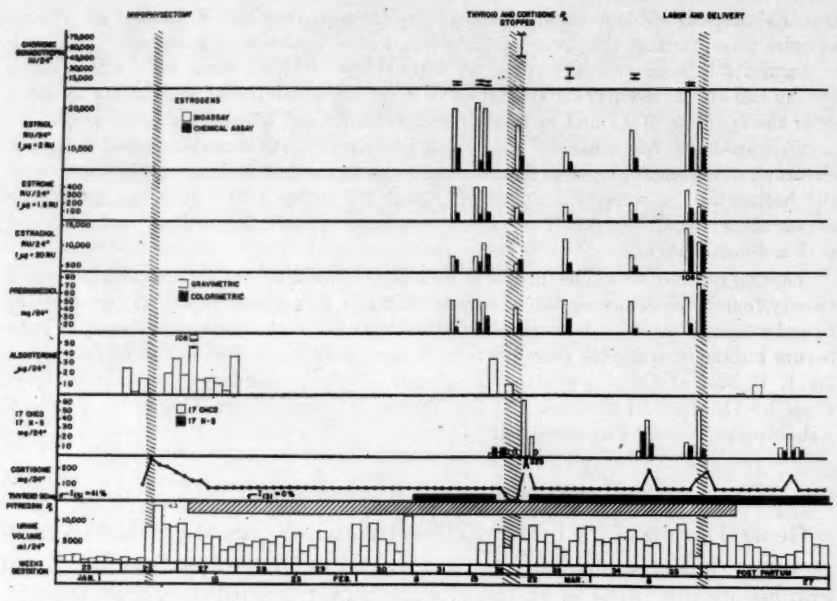

FIG. 1. Summary of urinary findings and hormonal therapy during the course of pregnancy from the twenty-fifth week of gestation to delivery (thirty-fifth week).

pophysectomy than during the following five days or the three days before operation.

Chorionic gonadotropin

The titer for urinary chorionic gonadotropin (C.G.) at 25 weeks was 20,000 to 30,000 I.U. per twenty-four hours. After hypophysectomy, at 28 weeks, the titer was normal—10,000 to 20,000 I.U. per twenty-four hours. During week 32, by both frog and rat assay methods, C.G. was maintained at a normal level of 4,000 to 8,000 I.U. per twenty-four hours. When thyroid was omitted for four days and cortisone for two days there was a tenfold increase in the output of C.G. After the re-institution of therapy, there was a gradual return to normal. Such an increase at this period of gestation does not occur in normal pregnancy (28) but has been found to precede clinical signs of placental deficiency (29, 30). In this patient, these clinical signs appeared in the form of premature uterine contractions starting at 34 weeks.

Sodium "pregnanediol" glucuronide complex

Pregnanediol output, whether based on the whole NaPG complex measured gravimetrically and calculated as pregnanediol[5] or on the color assay

for pregnanediol only, was within the range of normal during week 32. There was a slight drop in both values during omission of thyroid and cortisone, and the curves of excretion during the following four weeks failed to show the steady increase which characterizes pregnancies that progress normally to term (31, 32). As estimated gravimetrically, there is normally a two- to fourfold increase between week 30 and week 36 (31). There was no increase over the five-week period this patient was followed, until after the induction of labor.

Increased pregnanediol output during labor has sometimes been observed, but never to the degree noted in this patient. The question arose as to whether or not products of the cortisol given during labor were being measured in the NaPG complex and causing overestimates of pregnanediol in the color assays. Assays for ketosteroids in the 8 samples of NaPG complex indicated the presence of 20-ketosteroids only, *i.e.*, pregnanolone; and this was in normal amounts (8) with no increase during labor. Assays for 17-ketogenic steroids yielded negative results, indicating the absence of pregnane-3α,17α,20α-triol which, if present, would give falsely high titers in the pregnanediol color assay. The marked increase in NaPG complex and in its pregnanediol content during labor, therefore, appeared to reflect a true increase in pregnanediol output.

Estrogens (Table 1)

The excretion of urinary estrogens at 31 weeks was lower than that in normal pregnancy (31, 33). The level of estrogens, however, is of less clinical significance than the curve of excretion. As estimated by bio-assay, there is normally a three- to fivefold increase in the excretion of estriol between week 30 and week 36 (31). In this patient (starting with the specimen collected when thyroid and cortisone were withdrawn) there was a pronounced drop in urinary estrogens, largely attributable to decreased activity in the estriol fraction, which reached its lowest point one week later. This reduction in estrogens was associated with backache and painful uterine contractions. At 34 weeks, two weeks after re-institution of hormone therapy, the estrogenic activity of all three fractions had started to rise again and at 35 weeks, just before labor was induced, was back to the levels observed prior to interruption of replacement therapy. The subsequent drop in total estrogenic activity of the urine during active labor is a normal finding (31).

A large discrepancy may be noted between bioassay findings and chemical assay findings, particularly regarding the estrone and estriol fractions

[5] Steroidal products of progesterone other than pregnanediol are recovered in the NaPG complex and contribute to the gravimetric assay values (8).

(Table 1). More estrogen by bioassay than can be accounted for chemically has been observed in other cases ((9) and unpublished data[3]). Since the chemical procedure employed (10) has been shown to be quantitatively reliable for estradiol, estrone and estriol in pregnancy urine, it is apparent that estrogenic material not accounted for by these three classic estrogens was present in considerable amounts. Furthermore, the marked changes observed during the course of this study in the excretion of estrogenically active material were not accompanied by significant changes in the chemical values for estradiol, estrone and estriol. The bioassay results appeared to be correlated more closely throughout with the clinical picture and with the other hormonal findings.

High titers for chorionic gonadotropin accompanied by a drop in urinary estrogen and a drop (or failure to rise) in the curve of excretion of the NaPG complex have been frequently observed prior to the spontaneous onset of labor (whether at term or premature) and prior to such late pregnancy complications as toxemia or intra-uterine death of the fetus (29). In our patient, these considerations (together with a 3-cm. dilatation of the cervix) led to the decision that labor should be induced. Assays that revealed the subsequent rise in urinary estrogens and the return of chorionic gonadotropins to normal levels were not completed until after this decision had been made.

17-Hydroxycorticosteroids and 17-ketosteroids (Figs. 1, 2, 3 and 4)

The low urinary concentrations of 17-hydroxycorticosteroids in the thirty-first week of pregnancy dropped to zero when cortisone therapy was stopped (Fig. 2). The 17-ketosteroids fell from a normal level to 0.5 mg. per twenty-four hours on the second day without adrenocortical hormone therapy, and were zero the following day, in spite of the large doses of cortisone and cortisol which were then given. Plasma 17-hydroxycorticosteroids, which were normal while the patient was receiving 75 mg. of cortisone daily, dropped to zero on the two days in which cortisone was omitted. The first cortisol infusion was given on the morning of the third day (Fig. 2). The second cortisol infusion (Fig. 3) was given two weeks later; and the third, ten days post partum (Fig. 4). The apparent half-time ($t\frac{1}{2}$)[6] of corticoid disappearance from the blood was 123 minutes (no cortisone therapy)

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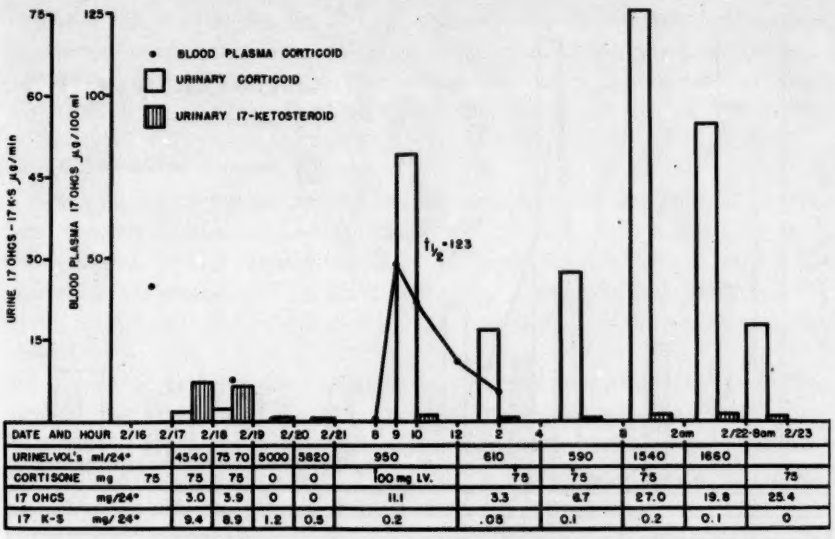
[6] $$t\frac{1}{2} = \frac{0.301}{K'}$$

where

$$K' = \frac{\Delta - \log N}{\Delta t}$$

where t = time and N = plasma value.



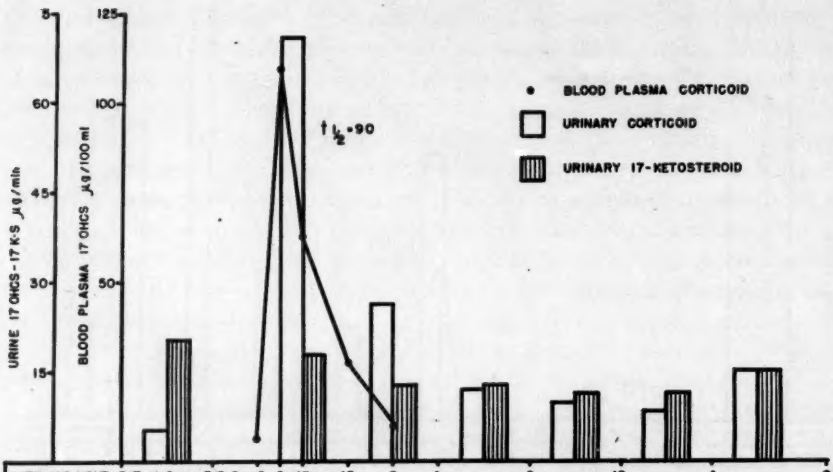
DATE AND HOUR	2/16	2/17	2/18	2/19	2/20	2/21	8	9	10	12	2	4	8	2am	2/22-8am	2/23
URINE-VOL's ml/24°		4540	75 70	5000	3820		950			610		590	1540	1660		
CORTISONE mg 75		75	75	0	0		100 mg I.V.			75		75	75			75
17 OHCS mg/24°		3.0	3.9	0	0		11.1			3.3		6.7	27.0	19.8		25.4
17 K-S mg/24°		9.4	8.9	1.2	0.5		0.2			.05		0.1	0.2	0.1		0

FIG. 2. Results of cortisol infusion test (100 mg. i.v.) after hypophysectomy during the thirty-second week of pregnancy when cortisone replacement therapy was stopped, showing levels of plasma and urinary 17-hydroxycorticosteroids and urinary 17-ketosteroids.

in the first test, 90 minutes in the second test (while receiving cortisone), and 105 minutes post partum (while receiving cortisone); but the one-hour peak plasma levels were quite different, 47.8 µg., 109.0 µg. and 141 µg. per 100 ml. respectively, in spite of the fact that the excretion per minute over the four-hour test period in each case was 50 µg., 71 µg. and 54 µg., respectively. The 17-ketosteroid values during the four-hour infusions were proportional to the 24-hour excretion values in all tests.

In the thirty-fourth week, while there was still demonstrable altered excretion of estrogens and pregnanediol and while the patient was receiving the same dosage of cortisone, urinary 17-hydroxycorticosteroids rose, though remaining within the normal range. 17-Ketosteroid excretion, however, was extremely high at this time—28.6 mg. per twenty-four hours. These values had changed by the thirty-fifth week to 19.8 mg. of 17-hydroxycorticosteroids and 15.5 mg. of 17-ketosteroids per twenty-four hours.

The urinary corticoid value (55.0 mg. per twenty-four hours) during labor reflected the infusion of cortisol. The 17-ketosteroids were normal but the rate of excretion for both was more than doubled in the second stage of labor. The urinary corticoids of the baby on days 2, 3 and 4 of its life were 78 µg., 142 µg. and 144 µg. per twenty-four hours.



DATE AND HOUR	3/7 at 8pm.	3/8, 8pm 8 9 10	12 2 4	8	12	4	9
URINE VOL's cc	5300	650	600	400	160	225	350
CORTISONE mg	75	100 mg I.V.			25		25
17 OHCS mg	7.8	17.0	6.2	3.0	2.5	1.9	4.6
17 K-S mg	28.6	4.6	3.3	3.3	2.8	2.8	4.6

FIG. 3. Results of cortisol infusion test (100 mg. i.v.) after hypophysectomy in the thirty-fifth week of pregnancy, during cortisone therapy, showing levels of plasma and urinary 17-hydroxycorticosteroids and urinary 17-ketosteroids.

The daily levels of blood and urinary 17-hydroxycorticosteroids directly depend upon the dosage of cortisone being administered. The rapidity with which these levels dropped to zero when cortisone was stopped indicated the complete absence of endogenous hormone. The urinary 17-ketosteroids are of much more complex origin. Although excretory levels of 17-ketosteroids dropped slowly to zero when cortisone was stopped, the high levels two weeks later remain unexplained. The very low levels or absence of endogenous 17-hydroxycorticosteroids make it likely that all adrenal secretory activity was reduced save that of aldosterone.

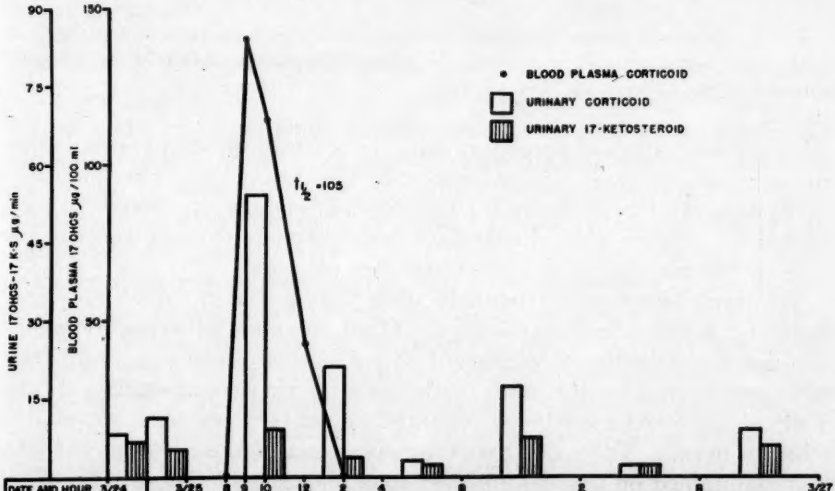
The apparent half-times ($t_{\frac{1}{2}}$) of plasma corticoid disappearance in each hydrocortisone infusion test were not markedly different (123, 90 and 105 minutes). The second test with the shortest half-time may have reflected the increased excretion of 17-hydroxycorticosteroids. The half-times of the two infusions administered during pregnancy were low compared to the delayed half-time expected for pregnancy (226 minutes) (34). The reasons for this difference are obscure, but depend not only on possible altered degradation and binding, but also on hemodynamic changes which were present.

The urinary excretion of 17-hydroxycorticosteroids in the first days of life of the infant followed the normal expected pattern, when it is remembered that during labor the mother was given a large dose of cortisol which would have crossed the placental membrane.

Thyroid (Table 2)

The 24-hour thyroidal uptake of radioactive iodine determined prior to hypophysectomy at the twenty-fourth week of gestation was 41 per cent; this value is within the normal limits obtained in this laboratory for the non-pregnant subject. The level of serum protein-bound iodine before hypophysectomy was 6.6 μg. per 100 ml.—at the lower limit of normal for pregnancy.

Unfortunately, the administration of potassium iodide as an expectorant for nine days starting on the first postoperative day invalidates the interpretation of the serum protein-bound iodine levels and thyroidal radioactive iodine uptakes determined over the subsequent weeks. It would be expected that these values would have fallen to levels indicative of panhypopituitarism had potassium iodide not been given (35). The fact that, de-



DATE AND HOUR	3/24		3/25	8	9	10	12	2	4	6	2	6	3/27
URINE VOL's cc	1050	1245	845				1000	130	850	285		3580	
CORTISONE mg	75		*100 mg I.V.				25		25			75	
17 OHCS mg	6.0	7.9	13.0				5.0	0.7	6.1	7.0		13.8	
17 K-S mg	5.2	3.8	2.1				1.0	0.5	2.7	1.7		9.3	

FIG. 4. Results of cortisol infusion test (100 mg. i.v.) ten days post partum during continuation of cortisone therapy, showing levels of plasma and urinary 17-hydroxycorticosteroids and urinary 17-ketosteroids.

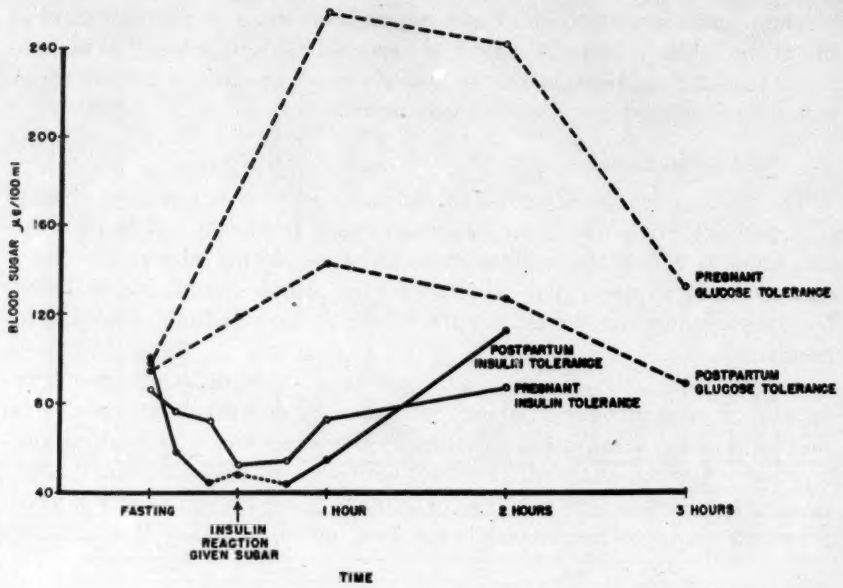


FIG. 5. Results of glucose tolerance and insulin tolerance tests during pregnancy (31 weeks) and post partum (5 days) while the patient was maintained on 75 mg. of cortisone and 90 mg. of desiccated thyroid daily.

spite administration of potassium iodide, the serum protein-bound iodine and butanol-extractable iodine levels at 31 weeks were low for normal pregnancy is suggestive evidence for thyroid deficiency, even though the patient was receiving what should have been adequate thyroid replacement therapy (90 mg. daily), and was clinically euthyroid.

The levels of serum thyroxine-binding protein (TBP), which are unaffected by iodide administration[7], remained elevated following hypophysectomy and at delivery, consistent with a normal pregnancy (36). They were not altered by the acute withdrawal of thyroid or cortisone. The TBP of umbilical cord blood obtained at delivery was also elevated—which is normal. Thus, in a pregnant woman without an hypophysis who was maintained on thyroid and cortisone replacement therapy, the TBP changes of normal pregnancy nevertheless were sustained. This observation supports the thesis that the rise in the level of serum TBP associated with normal pregnancy is not attributable to direct hypophyseal hormonal effects. In view of the rise in TBP level in non-pregnant patients with panhypopituitarism receiving diethylstilbestrol (37), it is of interest to

[7] Personal communication from Dr. N. Freinkel.

TABLE 2. THYROID STUDIES

Determination	Week of gestation								Umbil. cord blood	Values for non-pregnant normal subject	Values for 3rd trimester of pregnancy
	24	25	27	28	29	31	31*	35			
Thyroidal I^{131} uptake (% dose/24 hrs.)	41	†	0	—	—	—	—	—	—	15–50	? elevated
Serum protein-bound iodine (μg./100 ml.)	6.6		7.8	6.4			6.2	7.4	10.0	4.0–8.0	6.0–11.0
Serum butanol-extractable iodine (μg./100 ml.)		HYPOPHYSECTOMY				3.9				3.2–6.4	5.5–10.0
Serum total iodine (μg./100 ml.)							7.4	8.8	11.2	5.0–9.0	7.0–12.0
Serum thyroxine-binding protein (% radioactivity associated with)						85.0	83.2	87.4	73.9	60.6	92.8
Serum albumin (% radioactivity associated with)						12.2	10.2	7.6	19.0	34.5	7.2

* Thyroid and cortisone therapy had been stopped (see text).

† From 25 to 27 weeks' gestation, 10 gtts. of potassium iodide given t.i.d. for nine days and syrup of hydriodic acid, 4 cc. q.i.d. for three days.

speculate that the normal elevation of TBP concentration observed in this hypophysectomized pregnant patient might have been a response to placental estrogens.

Glucose and insulin tolerance (Fig. 5)

An oral glucose tolerance test at 31 weeks, before the interruption of thyroid and cortisone therapy, yielded a curve of the diabetic type (Fig. 5). An insulin tolerance test at this time failed to show the increased sensitivity that would be expected in a nonpregnant patient in the absence of the pituitary. Five days after delivery, however, with the same dietary preparation and the same dosages of thyroid and cortisone, the oral glucose tolerance curve was normal, and the same amount of insulin elicited the expected hypersensitive response and an insulin reaction.

It has been shown, in patients with normal pituitary function, that administered cortisone will reduce glucose tolerance (38) and increase insulin resistance (39). The postpartum findings in this hypophysectomized patient indicate, however, that 75 mg. of cortisone given daily as replacement did not have these effects and could not account for the prepartum observations. Normal pregnancy is characterized by increased resistance to insulin (40). This patient's unexpected resistance at 31 weeks could be explained on this basis, and would indicate that pituitary factors are not the cause of this alteration during pregnancy. The diabetic-like response to oral glucose at 31 weeks, however, remains unexplained. Here again the implication is that pituitary factors are not involved in such a response. The fact that it occurred during pregnancy but not post partum in this patient suggests

TABLE 3. ELECTROPHORETIC STUDIES OF SERUM

Subject	Albumin*	Globulins*				Tot. protein (Gm./100 ml.)
		Alpha 1	Alpha 2	Beta	Gamma	
Mrs. D.D.						
Feb. 16 (week 34)	45	7	10	24	13	5.3
Mar. 8 (week 34)	34	7	12	23	23	5.0
Mar. 15 Delivery	30	9	12	18	30	3.9
Controls [11]†	46	7	12	17	18	6.5
Baby D (cord blood)	49	11	10	6	24	5.6
Controls [11]	54	5	8	10	22	6.1

* Expressed as percentages of total protein.

† Figures in brackets denote the number of cases.

that her carbohydrate regulating mechanism, while 75 mg. of cortisone and 90 mg. of thyroid were being administered daily, was not entirely normal under the increased demands of pregnancy.

Electrophoretic patterns of serum proteins (Table 3)

The electrophoretic patterns during the last five weeks of pregnancy showed a remarkable alteration. At 31 weeks, prior to the interruption of replacement therapy, the total protein concentration and the pattern of distribution of protein fractions were normal. Between this time and the time of delivery at 35 weeks, the serum total protein level dropped from 5.3 to 3.9 Gm. per 100 ml., the albumin content fell from 45 to 30 per cent of the total, and the gamma globulin content rose from 13 to 30 per cent. The normal pregnancy elevation of α_2 globulin was maintained, however, and the β-globulin fraction, although it dropped from 24 to 18 per cent of the total, still was within the normal range.

It is possible that administration of cortisone or the presence of cancer may have influenced these values. However they are distinctly abnormal for this period of gestation, and the rapid development of this altered pattern during a period of no increase in cortisone dosage and no recrudescence of cancer, argues against this explanation. It may be pertinent that this change in the pattern of serum proteins had its beginning when cortisone and thyroid were omitted and that, coincident with this change, there appeared hormonal and then clinical evidence for failing placental function. That the α_2 and β globulins remained, after hypophysectomy, within the normal elevated range in pregnancy suggests that pituitary factors do not influence this pregnancy pattern.

Acknowledgments

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REFERENCES

1. White, T. T.: Carcinoma of the breast and pregnancy, *Ann. Surg.* **139:** 9–18, 1954.
2. Haagensen, C. D.: Carcinoma of the Breast. Series on the Early Recognition of Cancer, No. 5. Chicago, American Cancer Society, Inc., 1950, p. 82.
3. Smith, P. E.: Continuation of pregnancy in rhesus monkeys (Macaca mulatta) following hypophysectomy, *Endocrinology* **55:** 655–664, 1954.
4. Opsahl, J. C., and Long, C. N. H.: Identification of ACTH in human placental tissue, *Yale J. Biol. & Med.* **24:** 199–209, 1951.
5. Johnson, R. H., and Haines, W. G.: Evidence of small amounts of adrenal cortex hormone activity in human placenta, *Science* **116:** 456, 1952.
6. Smith, G. V., and Smith, O. W.: The anterior pituitary-like hormone in late pregnancy toxemia, *Am. J. Obst. & Gynec.* **38:** 618–624, 1939.
7. Venning, E. H.: Gravimetric method for the determination of sodium pregnandiol glucuronidate (an excretion product of progesterone), *J. Biol. Chem.* **119:** 473–480, 1937.
8. Smith, O. W., and Barker, C.: Factors influencing the composition of the sodium "pregnanediol" glucuronide complex in human pregnancy, with especial reference to diethylstilbestrol therapy, *J. Clin. Endocrinol. & Metab.* **12:** 152–168, 1952.
9. Smith, O. W., and Blackham, N. N.: The measurement of oestrogens in the urine of women, with special reference to hydrolytic procedures, *Acta endocrinol.* **25:** 133–160, 1957.
10. Brown, J. B.: A chemical method for the determination of oestriol, oestrone and oestradiol in human urine, *Biochem. J.* **60:** 185–193, 1955.
11. Smith, O. W., and Smith, G. V.: Endocrinology and related phenomena of the human menstrual cycle, *in* Recent Progress in Hormone Research, edited by Pincus, G. New York, Academic Press Inc., 1952, vol. 7, pp. 209–253.
12. Pincus, G., and Pearlman, W. H.: Fractionation of neutral urinary steroids, *Endocrinology* **29:** 413–424, 1941.
13. Nelson, D. H., and Samuels, L. T.: A method for the determination of 17-hydroxycorticosteroids in blood: 17-hydroxycorticosterone in the peripheral circulation, *J. Clin. Endocrinol. & Metab.* **12:** 519–526, 1952.
14. Glenn, E. M., and Nelson, D. H.: Chemical method for the determination of 17-hydroxycorticosteroids and 17-ketosteroids in urine following hydrolysis with β-glucuronidase, *J. Clin. Endocrinol. & Metab.* **13:** 911–921, 1953.
15. Drekter, I. J.; Heisler, A.; Scism, G. R.; Stern, S.; Pearson, S., and McGavack, T. H.: The determination of urinary steroids. I. The preparation of pigment-free

extracts and a simplified procedure for the estimation of total 17-ketosteroids, *J. Clin. Endocrinol. & Metab.* **12**: 55–65, 1952.

16. Hernando, L.; Crabbé, J.; Ross, E. J.; Reddy, W. J.; Renold, A. E.; Nelson, D. H., and Thorn, G. W.: Clinical experience with a physicochemical method for estimation of aldosterone in urine, *Metabolism* **6**: 518–543, 1957.
17. Zaffaroni, A.: Micromethods for analysis of adrenocortical steroids, *in* Recent Progress in Hormone Research, edited by Pincus, G. New York, Academic Press Inc., 1952, Vol. 8, pp. 51–86.
18. Bush, I. E.: Methods of paper chromatography of steroids applicable to the study of steroids in mammalian blood and tissues, *Biochem. J.* **50**: 370–378, 1952.
19. Nowaczynski, W.; Goldner, M., and Genest, J.: Microdetermination of corticosteroids with tetrazolium derivatives, *J. Lab. & Clin. Med.* **45**: 818–824, 1955.
20. Folin, O.; Todd, J. C. and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods. Philadelphia, W. B. Saunders Co., 1948, p. 383.
21. Swahn, B.: A method for localization and determination of serum lipids after electrophoretical separation on filter paper, *Scandinav. J. Clin. & Lab. Invest.* **4**: 98–103, 1952.
22. Zak, B.; Willard, H. H.; Myers, G. B., and Boyle, A. J.: Chloric acid method for determination of protein-bound iodine, *Analyt. Chem.* **24**: 1345–1348, 1952.
23. Venning, E. H.; Primrose, T.; Caligaris, L. C. S., and Dyrenfurth, I.: Aldosterone excretion in pregnancy, *J. Clin. Endocrinol. & Metab.* **17**: 473–482, 1957.
24. Martin, J. D., and Mills, I. H.: Aldosterone excretion in normal and toxaemic pregnancies, *Brit. M. J.* **2**: 571–573, 1956.
25. Llaurado, J. G.: Increased excretion of aldosterone immediately following operation, *Lancet* **1**: 1295–1299, 1955.
26. Luetscher, J. A.: Studies of aldosterone in relation to water and electrolyte balance in man, *in* Recent Progress in Hormone Research, edited by Pincus, G. New York, Academic Press Inc., vol. 12, 1956, pp. 175–198.
27. Bartter, F. C.; Liddle, G. W.; Duncan, L. E.; Barber, J. K., and Delea, C.: The regulation of aldosterone secretion in man: the role of fluid volume, *J. Clin. Invest.* **35**: 1306–1315, 1956.
28. Smith, O. W., and Smith, G. V.: Prolan and estrin in the serum and urine of diabetic and nondiabetic women during pregnancy, *Am. J. Obst. & Gynec.* **33**: 365–379, 1937.
29. Smith, G. V., and Smith, O. W.: Internal secretions and toxemia of late pregnancy, *Physiol. Rev.* **28**: 1–22, 1948.
30. Loraine, J. A., and Matthew, G. D.: Chorionic gonadotrophin in toxaemias of pregnancy, *J. Obst. & Gynaec. Brit. Emp.* **57**: 542–551, 1950.
31. Smith, O. W.; Smith, G. V., and Schiller, S.: Estrogen and progestin metabolism in pregnancy. I. Spontaneous and induced labor, *J. Clin. Endocrinol.* **1**: 461–469, 1941.
32. Michie, E. A.: Urinary pregnanediol excretion in normal pregnancy, *J. Obst. & Gynaec. Brit. Emp.* **60**: 561–564, 1953.
33. Brown, J. B.: Urinary excretion of oestrogens during pregnancy, lactation and the re-establishment of menstruation, *Lancet* **1**: 704–707, 1956.
34. Garst, J. B., and Assali, N. S.: Adrenal cortical capacity and cortisol utilization in normal and toxemic pregnancies, *Proc. Soc. Exper. Biol. & Med.* **92**: 815–819, 1956.
35. Li, M. C.; Rall, J. E.; Maclean J. P.; Lipsett, M. B.; Ray B. S., and Pearson,

O. H.: Thyroid function following hypophysectomy in man, *J. Clin. Endocrinol. & Metab.* **15**: 1228–1238, 1955.

36. Dowling, J. T.; Freinkel, N., and Ingbar, S. H.: Thyroxine-binding by sera of pregnant women, *J. Clin. Endocrinol. & Metab.* **16**: 280–282, 1956.
37. Dowling, J. T.; Freinkel, N., and Ingbar, S. H.: Effect of diethylstilbestrol on the binding of thyroxine in serum, *J. Clin. Endocrinol. & Metab.* **16**: 1491–1506, 1956.
38. Sprague, R. G.; Power, M. H.; Mason, H. L.; Albert, A.; Mathieson, D. R.; Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: Observations on the physiologic effects of cortisone and ACTH in man, *Arch. Int. Med.* **85**: 199–258, 1950.
39. Conn, J. W.; Fajans, S. S.; Louis, L. H., and Johnson, B.: Metabolic and clinical effects of corticosterone in man, *in* Proceedings of the Second Clinical ACTH Conference, edited by Mote, J. R. New York, the Blakiston Co., 1951, Vol. 1, pp. 221–251.
40. Burt, R. L.: Peripheral utilization of glucose in pregnancy. III. Insulin tolerance. *Obst. & Gynec.* **7**: 658–664, 1956.

LEVELS OF 17-HYDROXYCORTICOSTEROIDS AND 17-KETOSTEROIDS IN MATERNAL AND CORD PLASMA IN TERM ANENCEPHALY*†

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ABSTRACT

In plasma samples obtained from blood drawn simultaneously from the mother and the umbilical cord in 5 cases of term anencephalic infants the concentrations of 17-OH-CS were similar to those observed in normal infants, the cord levels being approximately half to a fourth the levels in the corresponding mother. Since the anencephalic infant has an atrophic adrenal devoid of fetal zone, it is concluded that 17-OH-CS in the normal infant are not produced by the fetal zone of the adrenal cortex. In 1 case of anencephaly, no 17-KS were detected in the plasma of either the mother or the umbilical cord. In another case the levels were lower than normal. A case of anencephaly is presented with a "normal" pharyngeal pituitary and with the usual atrophic adrenals, further suggesting that the fetal zone is not directly under the influence of the pituitary gland or that the pituitary is not secreting hormone.

IN RECENT years, many studies of the adrenal cortex of the fetus and newborn, both in man and in the experimental animal, have been reported. The literature on this subject has been thoroughly reviewed by Lanman (1), Gardner (2), and Davis and Plotz (3). The adrenal of the newborn infant consists of an outer subcapsular rim of cells which persists to form the definitive cortex of the adult and a much thicker inner layer of cells which immediately undergoes involution. This inner zone is known as the "fetal zone." With the exception of certain primates (4), this zone is found only in man, and its function has been the subject of much speculation. It is generally believed that the fetal zone is the site of synthesis of the 17-ketosteroids (17-KS) found in high concentrations in the plasma of newborn infants. The plasma titers of 17-hydroxycorticosteroids (17-OH-

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CS) in the first few days of life are low (5) and it has been suggested that these hormones are not formed by the fetal adrenal—the normal or moderately high levels observed at birth being the result of diffusion of steroids across the placenta from the maternal circulation (6).

It is well known that the anencephalic infant at 9 months' gestation has a defective adrenal cortex; the fetal zone is absent and the entire cortex is small, being composed of cells resembling those of a child 1 year of age. The determination of adrenal steroid levels in blood drawn simultaneously from the maternal circulation and the umbilical cord of an anencephalic infant at birth could help provide further information about the function of the fetal zone. The literature contains only the single observation (7) of a high 17-KS value in the plasma drawn twelve hours after the birth of an anencephalic infant (9 months' gestation) which lived twenty-five hours. The purpose of this paper is to report the 17-OH-CS and 17-KS values observed in plasma obtained simultaneously from maternal and cord circulation in 5 cases of anencephaly at term, and to discuss the results in relation to the possible function of the fetal adrenal cortex in the light of our current knowledge on this subject.

MATERIALS AND METHODS

Analyses of plasma

Blood was drawn into dry heparinized syringes from the median basilic vein of the mother immediately after delivery of the infant. The severed umbilical cord, before expulsion of the placenta, was allowed to bleed directly into heparinized centrifuge tubes. The blood was kept cool while being transported to the laboratory; the plasma was then separated and frozen.

The levels of plasma 17-OH-CS were determined by the method of Nelson and Samuels (8). In Cases 1, 2 and 3 the determinations were made in the laboratory of Isabel Taliaferro, Medical College of Virginia (mean normal value, 14.4 ± 6.2 micrograms (μg.) per 100 ml. of plasma); in Cases 4 and 5, the determinations were carried out in the Endocrine Research Laboratory of the Department of Pediatrics, The Johns Hopkins Hospital (mean normal value, 15.3 ± 5.0 μg. per 100 ml.).

A study of plasma 17-KS was carried out on blood samples from Cases 4 and 5, and several fractions were prepared:

a. *Free fraction.* The various plasma samples were extracted three times with once their volume of redistilled chloroform. The chloroform extracts were evaporated to dryness and the residues were chromatographed on a Florisil column. First, a 20-ml. chloroform fraction was discarded; then a 35-ml. fraction (2 per cent methanol in chloroform) was studied for its 17-KS content, and a 45-ml. fraction (25 per cent methanol in chloroform) was used for the determination of plasma 17-OH-CS. The fraction treated with 2 per cent methanol in chloroform was evaporated to dryness and applied on Whatman paper No. 2. The Bush system—Skellysolve C:methanol:water (100:85:15)—was used for chromatography of the paper (9), after which the paper was sprayed with the Zimmermann reagents.

b. *Glucuronoside fraction.* Following chloroform extraction, the proteins of the plasma

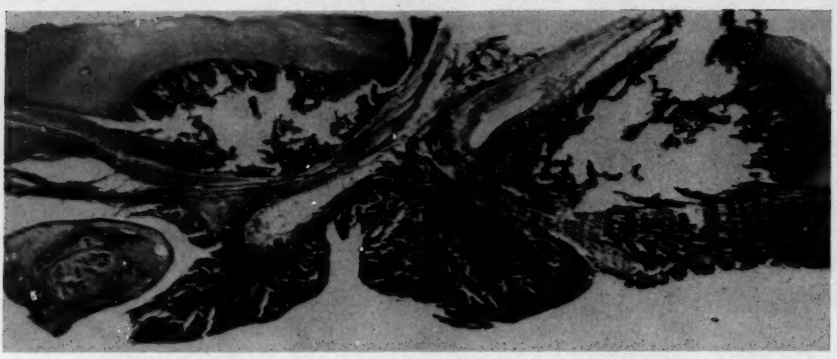

FIG. 1. *Sagittal section of sphenoid bone from Case 4 (anencephaly).* Note the disc-like pituitary on the pharyngeal surface immediately below the persistent cranio-pharyngeal canal. In the gross it measured 6 mm. in diameter and 1½–2 mm. in thickness. The wedge of free tissue anteriorly is an aberrant fold of nasal mucosa. Magnification 13×.

residues were precipitated with three times their volume of 100 per cent ethanol. After centrifugation, the aqueous solutions were decanted and the precipitates were washed with 30 ml. of 100 per cent ethanol. The pooled ethanol extracts were evaporated to dryness and the residues were taken into 25 ml. of water. Then 2 ml. of β-glucuronidase[1] and 2 ml. of 2 *M* acetate buffer were added, the pH of the mixture being adjusted to 4.6. The samples were incubated at 47° C. for fifteen hours, following which they were extracted three times with once their volume of chloroform. Chloroform extracts were evaporated to dryness and the residues were chromatographed on Florisil columns and on paper, as described for the free fraction.

c. *Hydrolysis at pH 0.8.* The pH of the plasma residues was brought to 0.8, and 48-hour continuous ether extractions were carried out. The ether extracts were evaporated to dryness and the residues were chromatographed as described for the free fraction. The areas of the paper chromatograms corresponding to dehydroepiandrosterone and androsterone were eluted and a Zimmermann reaction was run on the eluates, as described elsewhere (10).

Autopsies

All infants were delivered by the vaginal route at term. They died at birth, and shortly thereafter the autopsies were performed.

The adrenals were placed in 10 per cent formalin for twelve hours, after which the periadrenal fat was removed and the structures weighed. They were subsequently embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Pituitary tissue was not found in 4 of these infants; however serial sections were not made. The region of the sella turcica in each instance was carefully inspected with a dissecting microscope and in Case 4 a significant amount of pituitary tissue was found in the roof of the pharynx as a flat button-like protrusion. It was located at the base of the persistent craniopharyngeal canal (Fig. 1) and measured 6 mm. in diameter and 1½–2 mm. in thickness.

[1] Ketodase, a beef liver preparation (Warner-Chilcott Laboratories), was used in this study.

Sections of this pituitary tissue revealed an abundance of eosinophilic cells with a few basophilic and chromophobic cells in an edematous stroma of loose mesenchyme.

RESULTS

The adrenals had the small weight characteristic of the anencephalic infant (Table 1). It is interesting that the adrenals from Case 4, in which there was a pharyngeal pituitary, were the smallest of the lot, weighing 0.39 Gm. The adrenals from this case are shown in Figure 2 in comparison with adrenals weighing 7.2 Gm. from a "normal" newborn infant. A section of an adrenal from this "normal" infant is shown in Figure 3; it may be seen that the fetal zone comprises the major portion of the cortex. In all 5 cases of anencephaly, microscopic examination of the adrenals revealed no fetal zone; the glands consisted entirely of definitive cortex, as shown in the section from Case 4 (Fig. 4).

The levels of plasma 17-OH-CS are shown in Table 1. The values for the anencephalic infants were half to a fourth those for the corresponding mothers. A similar ratio, as well as similar values, have been reported for normal infants born by vaginal delivery (6).

FIG. 2. *Adrenals on left from a full-term "normal" infant dying two hours after birth with atelectasis* (combined weight 7.2 Gm.). These glands consist mainly of fetal zone. *Adrenals on right from Case 4 (anencephaly).* The fetal zone is entirely absent and the cortical tissue present is the rim of definitive cortex characteristic of anencephaly (combined weight 0.39 Gm.).

TABLE 1. WEIGHT OF ADRENALS AND LEVEL OF PLASMA 17-OH-CS

	Case 1, ♀		Case 2, ♀		Case 3, ♂		Case 4, ♂		Case 5,* ♂	
	Plasma 17-OH-CS (μg./100 ml.)	Combined adrenal weights (Gm.)	Plasma 17-OH-CS (μg./100 ml.)	Combined adrenal weights (Gm.)	Plasma 17-OH-CS (μg./100 ml.)	Combined adrenal weights (Gm.)	Plasma 17-OH-CS (μg./100 ml.)	Combined adrenal weights (Gm.)	Plasma 17-OH-CS (μg./100 ml.)	Combined adrenal weights (Gm.)
Maternal	40.6	—	51.7	—	44.0	—	25.8	—	38.4	—
Fetal	10.2	0.51	14.0	0.45	19.0	0.55	11.6	0.39	13.2	0.41

* Fetal heart beats inaudible two hours prior to delivery and not detected at birth.

No 17-KS were detected in the free fraction of the various plasma samples, except in the cord plasma of Case 5, in which there was possibly a spot corresponding to androsterone; the amount of material present could be estimated to be 2–3 μg., which would yield a concentration of 11.5–17.0 μg. per 100 ml. of plasma. A spot which absorbed ultraviolet light and exhibited a transient lavender color with the Zimmermann reagent was observed in all cases. However, the amount of material was proportionally two to three times greater in the cord samples than it was in the maternal samples. The Rf values of this spot were 0.389 to 0.405. It is interesting that, in contrast with the purple color produced by the 17-ketones with the Zimmermann reagent, the 4-3-ketones such as testosterone and progesterone produce a transient lavender color and that the Rf value of pure progesterone in the system of solvents used in this study is 0.399 to 0.404.

The paper chromatograms of the glucuronoside fractions did not reveal the presence of any 17-KS. A spot absorbing ultraviolet light, exhibiting a transient lavender color with the Zimmermann reagents, and having an Rf value of 0.419 was found in the cord samples of Cases 4 and 5; the amount of material appeared to be smaller than that observed in the free fraction. This spot was not detected in the chromatograms of maternal extracts.

In plasma treated by continuous extraction at pH 0.8, no androsterone could be detected in any sample. No dehydroepiandrosterone was found in samples from Case 4. In Case 5 the dehydroepiandrosterone concentration in maternal plasma was 1.8 μg. per 100 ml. and that in cord plasma was 4.6 μg., but such values are probably not significant. The eluate of the area between androsterone and dehydroepiandrosterone did not contain any 17-KS.

DISCUSSION

The absence of the fetal zone of the adrenal cortex in the anencephalic fetus has been ascribed to a deficient pituitary. This may not be entirely correct, since Angevine (11) in a careful study of several cases of anen-

cephaly involving serial sections of the sella region always found varying amounts of pituitary tissue. Our failure to find pituitary tissue in 4 of our 5 cases was probably due to the fact that we did not make serial sections of the region. The pharyngeal pituitary in Case 4 is the greatest amount of pituitary tissue so far reported in anencephaly. The small adrenals without a fetal zone in this case would seem to indicate that either this zone is not under the influence of pituitary tropic hormones, or that the pituitary is not functional. It is also possible that the absence of hypothalamus, regardless of the amount of pituitary tissue present, accounts for the lack of fetal zone in anencephaly.

The adrenal cortex of the anencephalic infant is formed normally up to at least the fifth month of gestation (12–14) even though the defect in the

FIG. 3. *Section of adrenal from the "normal" infant represented in Figure 2.* The definitive cortex can be seen as a thin rim of deeply staining cells beneath the capsule. The main mass of poorly staining cells is the fetal cortex, which will immediately undergo involution. Hematoxylin and eosin. Magnification 28×.

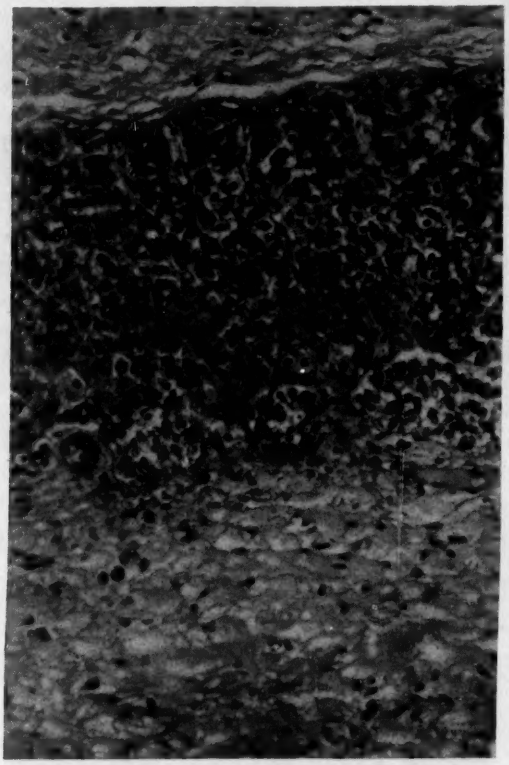

FIG. 4. *Section of adrenal from Case 4 (anencephaly).* The entire cortex consists of deeply staining cells similar to those which persist to form the definitive cortex in the normal gland. The inner space adjacent to the medulla is devoid of fetal cortex and is formed of edematous loose mesenchyme. Hematoxylin and eosin. Magnification 130×.

pituitary is present long before this time. This might indicate that in the normal fetus the adrenocorticotropic hormone which appears in the pituitary at the sixteenth week (15) simultaneously with the appearance of basophil cells (16) does not directly account for the formation of the fetal zone. This zone is often compared with the X-zone of the mouse, which is under the influence of gonadotropins. For this and other reasons, Chester Jones (17) has set forth the suggestion that the fetal cortex may be under the influence of chorionic gonadotropins. However, it is not possible at present to decide what mechanism controls the formation of the fetal zone.

The newborn infant has been reported to have high titers of androgenic steroids (18–20) followed by a rapid decline in the few first weeks of life

while the fetal zone is atrophying, suggesting that this zone is the site of androgen synthesis. The decrease in plasma 17-KS levels noted in Cases 4 and 5 of this study would tend to substantiate this hypothesis. The findings of Davis *et al.* (21) of a high rate of cholesterol synthesis in the normal adrenal cortex at 5 months' gestation, and a low rate in the glands of anencephalic infants, would also tend to confirm this theory. However, DiGeorge *et al.* recently reported that an anencephalic fetus at 9 months' gestation had a high titer of 17-KS in plasma (7). It is difficult to reconcile our findings with those of DiGeorge and collaborators, but it is evident that no definitive conclusion can presently be drawn from a study of a total of only 3 cases.

It is generally accepted that pregnancy (22–23) and labor (6) cause a rise in the level of maternal plasma 17-OH-CS; a similar elevation was observed in the present study. The ratio of cord to maternal values in our 5 cases of anencephaly was similar to that observed in normal cases of vaginal delivery (6). Migeon *et al.* (6, 24) demonstrated that 4-C^{14}-cortisol, as well as the non-isotopic steroid, can cross the "placental barrier," but in a definite proportion: these authors interpreted their finding to mean that the cord levels of plasma 17-OH-CS are merely the reflection of those of the mother and that the fetal adrenal gland near term produces little or no corticosteroids. However, it seems that the fetal adrenal gland can synthesize such steroids, since Bloch *et al.* (25) recovered C_{19}, C_{21} and sodium-retaining substances from fetal adrenal tissue. The fact that cord levels of plasma 17-OH-CS are lower than maternal levels and vary directly with exogenously administered hormone to the maternal organism does not exclude the possibility that the fetal adrenal gland may secrete these steroids at a constant low basal level. The finding of normal values of 17-OH-CS in anencephalic infants, however, precludes the possibility that these hormones are secreted by the fetal zone.

Acknowledgment

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REFERENCES

1. Lanman, J. T.: The fetal zone of the adrenal gland. Its developmental course, comparative anatomy and possible physiological functions, *Medicine* **32**: 389, 1953.
2. Gardner, L. I.: Adrenocortical metabolism of the fetus, infant and child, *Pediatrics* **17**: 897, 1956.
3. Davis, M. E., and Plotz, E. J.: Hormonal interrelationships between maternal adrenal, placental and fetal adrenal functions, *Obst. & Gynec. Surv.* **11**: 1, 1956.
4. Lanman, J. T.: The adrenal fetal zone: its occurrence in primates and a possible relationship to chorionic gonadotropin, *Endocrinology* **61**: 684, 1957.

5. KLEIN, R.; FORTUNATO, J., and PAPADATOS, C.: Free blood corticoids in the newborn infant, *J. Clin. Invest.* **33**: 35, 1954.
6. MIGEON, C. J.; PRYSTOWSKY, H.; GRUMBACH, M. M., and BYRON, M. C.: Placental passage of 17-hydroxycorticosteroids: comparison of the levels in maternal and fetal plasma and effect of ACTH and hydrocortisone administration, *J. Clin. Invest.* **35**: 488, 1956.
7. DIGEORGE, A. M.; AREY, J. B., and BONGIOVANNI, A. M.: Plasma 17-ketosteroids in an anencephalic infant, *J. Clin. Endocrinol. & Metab.* **16**: 1281, 1956.
8. NELSON, D. H., and SAMUELS, L. T.: A method for the determination of 17-hydroxycorticosteroids in blood: 17-hydroxycorticosterone in the peripheral circulation, *J. Clin. Endocrinol. & Metab.* **12**: 519, 1952.
9. BUSH, I. E.: Methods of paper chromatography of steroid applicable to the study of steroids and mammalian blood and tissues, *Biochem. J.* **50**: 370, 1952.
10. MIGEON, C. J., and PLAGER, J. E.: A method for the fractionation and measurement of 17-ketosteroids in human plasma, *J. Clin. Endocrinol. & Metab.* **15**: 702, 1955.
11. ANGEVINE, D. M.: Pathologic anatomy of hypophysis and adrenals in anencephaly, *Arch. Path.* **26**: 507, 1938.
12. MEYER, R.: Nebennieren bei Anencephalie, *Virchows Arch. f. path. Anat.* **210**: 158, 1912.
13. NICHOLS, J.: Observations on the adrenal cortex of the premature anencephalic fetus, *Arch. Path.* **62**: 312, 1956.
14. BENIRSCHKE, K.: Adrenal in anencephaly and hydrocephaly, *Obst. & Gynec.* **8**: 412, 1956.
15. TAYLOR, N. R. W.; LORAINE, J. A., and ROBERTSON, H. A.: The estimation of ACTH in human pituitary tissue, *J. Endocrinol.* (British) **9**: 23, 1953.
16. PEARSE, A. G.: Cytological and cytochemical investigations on the foetal and adult hypophysis in various physiological and pathological states, *J. Path. & Bact.* **115**: 355, 1953.
17. CHESTER JONES, I.: The Adrenal Cortex. Cambridge University Press, 1957.
18. GARDNER, L. I., and WALTON, R. L.: Plasma 17-ketosteroids of the human fetus; demonstration of a concentration gradient between cord and maternal circulation, *Helvet. paediat. acta* **4**: 311, 1954.
19. GARDNER, L. I.; WALTON, R. L.; ELLIS, W., and HUGHES, E. C.: Diminished plasma 17-ketosteroid concentration in pregnancy, *Proc. Soc. Exper. Biol. & Med.* **86**: 804, 1954.
20. MIGEON, C. J.: Dehydroepiandrosterone and androsterone levels in maternal and cord plasma, *in* Adrenal Function in Infants and Children, edited by L. I. Gardner. New York, Grune & Stratton, 1955.
21. DAVIS, M. E.; PLOTZ, E. J.; LEROY, G. V.; GOULD, G. S., and WERBIN, H.: Hormones in reproduction, *Am. J. Obst. & Gynec.* **72**: 740, 1956.
22. GEMZELL, C. A.: Blood levels of 17-hydroxycorticosteroids in normal pregnancy, *J. Clin. Endocrinol. & Metab.* **13**: 898, 1953.
23. ASSALI, N. S.; GARST, J. B., and VOSKIAN, J.: Blood levels of 17-hydroxycorticosteroids in normal and toxemic pregnancies, *J. Lab. & Clin. Med.* **46**: 385, 1955.
24. MIGEON, C. J.; BERTRAND, J., and WALL, P. E.: Physiological disposition of 4-C^{14}-cortisol during late pregnancy, *J. Clin. Invest.* **36**: 1350, 1957.
25. BLOCH, E.; BENIRSCHKE, K., and ROSEMBERG, E.: C_{19} steroids, 17-α-hydroxycorticosterone and a sodium retaining factor in human fetal adrenal glands, *Endocrinology* **58**: 626, 1956.

DAY-TO-DAY FLUCTUATION IN EXCRETION OF HUMAN PITUITARY GONADOTROPIN*

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ABSTRACT

Ten consecutive 24-hour specimens of urine from a healthy postmenopausal woman were collected, and assays of urinary gonadotropin were conducted with a suitable standard. A twofold day-to-day fluctuation in the excretion of gonadotropin was found, showing that excretion of gonadotropin in the postmenopausal state is not constant.

PREVIOUS data (1) on the excretion of human pituitary gonadotropin (HPG) in postmenopausal women led to the inference that the daily excretion was not constant. Furthermore, daily consecutive assays of HPG over long periods (two years in one instance) showed a pattern that appeared to be a combination of fortuitous fluctuations, different levels of excretion, and cyclic variations in excretion. The magnitude of the daily fluctuation, when measured as the span between the lowest and highest values, varied from a few per cent to tenfold. Since these assays were performed without a concomitant standard, the values are subject to methodologic error, the magnitude of which is not known.[1] Because extreme variations of this order of magnitude could result from methodologic error alone, it is uncertain that a cyclic pattern of HPG excretion actually occurs or, indeed, that a real daily fluctuation is present.

Some sources of error and an empiric estimation of its magnitude already have been given (1). Additional data will be presented here from which the extreme error of the routine clinical assay of HPG can be calculated. It became possible, therefore, to determine the effect of this error on the pattern of daily excretion. This determination was accomplished by testing the same quantity of gonadotropin each day, using the same procedure employed in the study of the daily excretion of HPG in postmenopausal or castrated women. If the excretion of gonadotropin in postmenopausal women were constant and if the resulting pattern were due entirely to the methodologic error of the assay, the same pattern would be obtained for

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[1] The precise error or even an appraisal of its order of magnitude is not known for any clinical assay method for HPG that uses animal units of one sort or another and that is carried out without a standard reference material. A sufficient supply of a suitable standard would make possible direct determination of the inherent error of any method.

both the identical daily dose of gonadotropin and the consecutive daily specimens of urine from a subject under study.

Although not ideal, this approach would lead to more definitive conclusions than were possible from previous studies. The ideal study would be one in which daily consecutive assays of 24-hour samples of urine from various persons were carried out for a prolonged period with an appropriate standard. The length of the present study was limited by the small amount of available standard (AMW) prepared from the urine of normal men (2). Ten consecutive 24-hour specimens of urine from a postmenopausal woman were assayed with this standard. Although the period of observation was short, ten days were deemed sufficient to ascertain the precise extent of the daily fluctuation and possibly the presence of any cyclic behavior.

MATERIAL AND METHODS

A 53-year-old normal healthy woman whose menses had ceased spontaneously three years previously, collected 10 consecutive 24-hour specimens of her urine. The daily specimens were processed as previously described (3), with the exception that the final 66 per cent acetone precipitate was dried and weighed. The precipitate then was dissolved in 24 ml. of water, each milliliter containing the equivalent of one hour's flow of urine. For each assay, 4 doses of the concentrate of a 24-hour specimen of urine and 2 doses of standard (4-and-2 design) were tested. The material from the daily specimens of urine was appropriately diluted so that 4 rats each received a half-hour equivalent of urine, 4 rats each received a one-hour equivalent, 3 rats a two-hour equivalent, and 3 rats a four-hour equivalent. The doses of the standard were 10 and 20 mg. per animal, each dose being given to a group of 5 animals. Injections were carried out over a four-day period, as previously described (3). The timing of the injection schedule was arranged to coincide exactly with that of our routine procedure for clinical assays of HPG.

The schedule for processing and injection was as follows: The 24-hour specimens of urine (8 A.M. to 8 A.M.) collected on Saturday, Sunday and Monday were individually processed on Monday, the injections being started on Monday. Urine collected on Tuesday was processed and injections were begun on the same day. Urine collected on Wednesday was processed that day, but the injections were begun on Thursday, so that necropsy of animals on Sunday was avoided. Urine collected on Thursday and Friday was prepared and injected on these respective days. Although the 4-and-2 design of assay circumvents the factor of daily spontaneous fluctuation in animal sensitivity, this plan of duplicating the routine procedure appeared worth while, especially since it had the further advantage of conserving the standard reference substance.

RESULTS

The mean ovarian weights corresponding to the 4 doses of concentrate from each of the 10 consecutive 24-hour specimens of urine and to the doses of standard are listed in Table 1, which includes urinary volumes and the weights of the gonadotropic concentrate. The relative potency of the daily specimens of urine in terms of the standard was calculated by statistical methods (4); the values for the index of precision (lambda), combined

slope (b), variance (s), relative potency, and confidence limits for P=0.95 are listed for each of the 10 assays.

DISCUSSION

The sources of methodologic error become evident when the clinical assay is examined. The numerical values (rat units of HPG per twenty-four hours) were obtained by a method of assay that, by necessity, involved determination of the mean ovarian weight of 2 rats (each treated with a 12-hour equivalent of urine) and the conversion of the mean ovarian weight into units per twenty-four hours by a calibration curve of fixed slope and position. The manner in which this curve of fixed slope and position was established is fully described in a previous publication (1). A certain response (mean ovarian weight of 22.5 mg.) was assigned a value of 5 rat units.[2] From the curve constructed relating response to dose, it was then possible to obtain units for any ovarian response measured as mean ovarian weight ranging from 22.5 mg. to 250 mg. The units corresponding to the mean ovarian weight obtained were, therefore, determined from the curve and multiplied by 2 (since a 12-hour equivalent of urine was administered

[2] The designation of 10 mg. of AMW as 5 rat units is partly arbitrary since, in general, any weight of a standard may be designated any number of units. However, in this instance, the assignment of 1 rat unit per 2 mg., or 1 mg. = 0.5 rat unit, was based on other expedient grounds. Prior to the institution in 1953 of the kaolin-acetone method of preparation and the use of the rat ovarian weight as the end point for clinical assays in this laboratory, the method employed was that of Frank and Salmon (*Proc. Soc. Exper. Biol. & Med.* **32**: 1237, 1935; **33**: 311, 1935; **34**: 804, 1936). In this latter method, "normal" values were said to be 5 rat units per liter, and "abnormal" values were said to be 25 or more rat units per liter. When the conversion of methodology was made, the problem arose whether to initiate a new and different numerical system of units or to arrange the new numerical values to correspond with the older familiar units. Since it appeared likely that an international standard preparation eventually would be established, the probability that still another change would be necessary in the near future had to be considered as well. Consequently, it was decided to arrange the new numerical values to correspond with the older familiar ones, and thus only one change would have to be made to a new and final system of units when conversion to international units became feasible. Accordingly, 10 mg. of the local standard AMW was assigned a value of 5 rat units, since this amount generally produced a mean ovarian weight of 22.5 mg., a 50 per cent increase over the control weight of 15 mg. The amount of gonadotropin per day in the urine of normal adult men and of young normal women (15 to 30 years of age) in general gave rise to responses, in terms of mean ovarian weight of rats, of from 20 mg. to less than 100 mg. Mean ovarian weights between 22.5 and 100 mg. correspond, therefore, to 5 to 25 rat units per day (see Figures 6 and 7, in reference 1). Thus, the resulting numerical values were the same as those in the Frank and Salmon method, and no change was necessary in the system of reporting values to which many clinicians utilizing gonadotropin assays had become accustomed. The only change was from rat units per liter to rat units per day—a minor adjustment that caused no confusion.

TABLE 1. CONSECUTIVE DAILY EXCRETIONS OF PITUITARY GONADOTROPIN IN A POSTMENOPAUSAL WOMAN

Day	Urinary volume (ml.)	Gonadotropic concentrate (mg.)	Mean ovarian weight (mg.)						λ*	b*	s*	Relative potency, u/s*	Confidence limits, P = 0.95
			Equivalent of urine tested				Standard						
			0.5 hr.	1 hr.	2 hrs.	4 hrs.	10 mg.	20 mg.					
1	1505	56.1	23	50	82	88	22	46	.14	85	11	9.3	(6.4–13.9)
2	2070	57.8	22	61	67	108			.11	105	11	8.8	(6.7–12.3)
3	2990	71.8	23	60	97	119	18	64	.06	144	8	6.7	(5.8– 7.8)
4	1810	77.4	18	44	74	124			.03	125	4	5.3	(4.7– 5.6)
5	2300	71.8	17	52	100	111	17	66	.04	156	7	6.0	(5.4– 7.1)
6	1800	70.2	26	52	72	91			.09	125	12	6.1	(4.9– 8.1)
7	2015	64.1	35	56	79	112	20	53	.11	90	10	10.0	(7.4–14.3)
8	1435	46.9	16	35	68	118	16	51	.11	90	10	8.3	(6.0–10.9)
9	1600	58.7	14	36	82	116			.07	95	7	6.4	(5.4– 8.0)
10	2400	68.5	17	42	68	80	19	57	.07	105	7	5.6	(4.8– 6.9)

* Abbreviations: λ = index of precision; b = combined slope; s = variance; u/s = the unknown (daily 24-hour specimen) ÷ the standard.

to each rat) to yield units of HPG per twenty-four hours. However, the ovarian response obtained to any dosage level of HPG is not fixed. The variability of response constitutes a major source of methodologic error. Other major sources of error are: 1) variation due to use of 2 animals per assay, 2) the effect of slope on the conversion of ovarian weight into units, 3) variation in slope of the dose-response line, 4) variation in sensitivity of the animals, and 5) the asymptotic nature of high ovarian weights.

The contribution of some of the sources of the methodologic error to the apparent day-to-day fluctuation in HPG excretion of postmenopausal women has been estimated. It was found that the mean ovarian weight of groups of 2 animals each did not vary more than approximately ±30 per cent from the mean ovarian weight of a group of 16 animals (a more acceptable number) given the same dose of HPG. This dose of HPG produced a mean ovarian weight of approximately 50 mg. It was also determined experimentally that the ±30 per cent deviation occurred at any mean ovarian weight from 25 mg. to 250 mg. Therefore, a deviation of ±30 per cent may be assumed, as an approximation, to represent an extreme deviation independent of the magnitude of the ovarian response. When ovarian weight is converted into units per day, the extreme variation can be calculated as follows. At a mean ovarian weight of 25 mg., a ±30 per cent deviation would result in an extreme variation of from 9 to 12.6 units per twenty-

four hours, a 1.5-fold difference between the lower and the higher value. At a mean ovarian weight of 50 mg., the extreme deviation would be from 13 to 27 units, a twofold span; at a mean weight of 100 mg., the extreme fluctuation would be fourfold, and at 150 mg. it would be eightfold. There appears to be no reason to assume that deviations in mean ovarian weight at any dose are not normally distributed. Therefore, extreme deviations of ± 30 per cent would be balanced by a small number of much smaller deviations. The remainder would represent deviations closer to the mean deviation, determined in a small number of trials to be about ± 15 per cent (1).

If the daily excretion of gonadotropin in postmenopausal women were constant, the curve obtained by plotting the daily values would constitute a pattern characteristic of random fluctuations of methodologic origin, containing several intermittent spikes of moderate proportions, a few wide excursions, and a few small excursions. Experimental verification that such a pattern results was obtained by testing 10 to 16 equal and accurately weighed samples of a gonadotropic preparation. A sample was dissolved each day for ten to sixteen days, and the four-day injection schedule was started as described for the routine method, with the same timing schedule as previously noted. This experiment was repeated with different doses to cover the entire range of ovarian response of mean weights ranging from 25 to 250 mg.

When the mean ovarian weight at a small constant dosage (response equal to a mean ovarian weight of 25 mg.) was plotted, the curve consisted of several irregular spikes, and a few wider and a few smaller excursions above and below an average line. For larger doses (mean ovarian weight of 150 mg.), the same pattern resulted, except that the excursions were greater in magnitude. When the mean ovarian weights for the period of ten to sixteen days were plotted against time as the logarithm of mean ovarian weight, the curves for both small and large doses were similar in magnitude of excursions, showing that the mean deviation in per cent was a constant proportion of the ovarian response and hence of the dose.

When the mean ovarian weights were converted into rat units per day by the use of the fixed-slope method, the "daily excretion" for the small constant dosage was converted to almost a straight line. This is an artifact. The resulting small numbers (in terms of units per day) in the first log cycle of the dose-response line allow for only small excursions when expressed as a span between low and high values. When the ovarian weight at large dosages was transformed into units, the magnitude of the excursions was large, a ninefold span being present between the lowest and the highest value.

Thus, the pattern obtained when the same dose of gonadotropin was administered daily was one of random deviations about a mean level, with the magnitude of the span being proportional to the dose. The pattern ob-

served in several studies conducted on persons over long periods differs from the artificial curves obtained with a constant dosage, for irregular cycles of excretion are present in addition to random fluctuations due to inherent error. Thus, the pattern in the present study appeared to be a resultant of at least 2 forces, namely, randomness due to methodologic error and cycles of activity indicative of inconstant HPG excretion.

The data based on 10 consecutive daily assays of HPG excretion in a postmenopausal woman, conducted with a simultaneous standard, provide direct evidence that the excretion of HPG is not constant. The effect of the use of a simultaneous standard is shown in Figure 1. The first 3 curves in this figure represent the pattern obtained when only the mean ovarian weight corresponding to a 1-hour equivalent of urine, a 2-hour equivalent or a 4-hour equivalent, respectively, was converted into units per day, as is done in routine assays without a standard. Patterns of daily excretion similar to those reported previously were obtained. Furthermore, it can be seen that the magnitude of the span between lowest and highest values is directly proportional to the dose, as predicted and experimentally verified.[3] The fourth curve in the figure represents the daily excretion when the assays were performed with a simultaneous standard. The values in rat units per day were obtained from the data in Table 1. Since 10 mg. of AMW is defined as 5 rat units,[2] the relative potency of each of the daily specimens multiplied by 0.5 and by the weight of the 24-hour concentrate gives the result in rat units per day. The pattern of excretion is now minimally contaminated with fluctuations of methodologic origin, yet it shows a twofold variation. That a twofold variation is highly significant is shown by transformation of the confidence limits in terms of the per cent of relative potency. Upper and lower confidence limits at $P = 0.95$ now can be expressed in terms of rat units by multiplying their values in per cent by the points in the fourth curve. No overlap occurred in confidence limits of 5 of the assays, showing a high likelihood that HPG excretion is not constant day by day. Whether the extent of the fluctuation is twofold, as in the woman studied, or is much larger or smaller can be decided only by similar studies in many persons. Presumably, it will vary in different individuals.

The occurrence of irregular cycles of three to five days in length at varying levels of HPG excretion in the same person followed for as long as two years was not obvious in this ten-day period. Only one such cycle was present. Whether such cycles occur with regularity or with any given fre-

[3] The mean level of excretion for the 10-day interval is approximately the same no matter how the assay is calculated. The mean excretion $\pm$ the standard deviation (S.D.) in rat units per day was 226 ± 43 for the 1-hour equivalent, 228 ± 62 for the 2-hour equivalent, 221 ± 64 for the 4-hour equivalent, and 228 ± 39 for the four-and-two assay. As stated previously, the standard deviation has increasing magnitude when the assay is calculated at higher ovarian weights in the one point-fixed slope method.

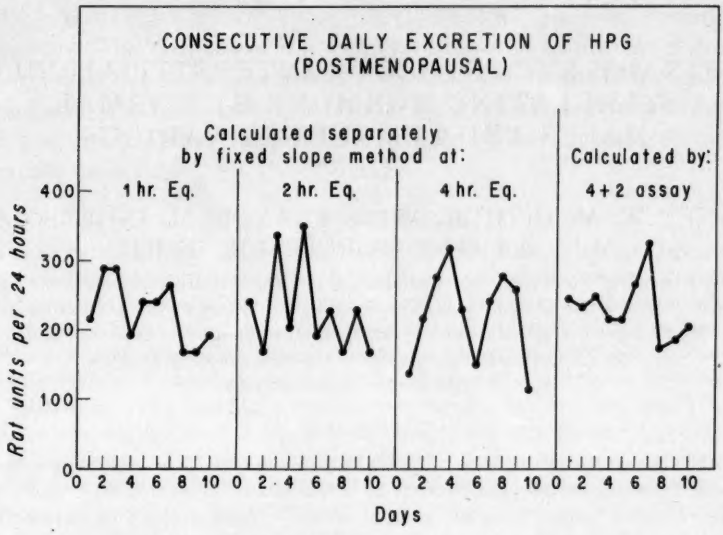

FIG. 1. Plots of the consecutive daily excretion of gonadotropin in a postmenopausal woman (see text for details).

quency also can be determined only by assays with a standard over much longer periods—a project that cannot be undertaken until a sufficient supply of standard is available.

Apart from the main aspect of this study, the data show how necessary is the use of a standard for meaningful interpretation of assays of gonadotropin in studies of human pituitary gonadotropic function. For example, a single one-point assay before and a single one-point assay after the administration of a steroid could yield meaningless or misleading results. Because of the lack of a standard, it has been necessary to perform many daily consecutive one-point assays in order to achieve a reasonable estimate of a control base line (1). However, with a standard, the control period can be sharply reduced and accurate assays obtained, thus facilitating clinical investigations in this field.

REFERENCES

1. ALBERT, A.: Human urinary gonadotropin, *in* Recent Progress in Hormone Research, ed. by Pincus, G. New York, Academic Press Inc., 1956, vol. 12, pp. 227–301.
2. ALBERT, A.: Preparation of a provisional standard for urinary gonadotropin of men, *Proc. Staff Meet., Mayo Clin.* **31:** 341–344 (May 30) 1956.
3. ALBERT, A.: Procedure for routine clinical determination of urinary gonadotropin, *Proc. Staff Meet., Mayo Clin.* **30:** 552–555 (Nov. 16) 1955.
4. BORTH, R.; DICZFALUSY, E., and HEINRICHS, H. D.: Grundlagen der statistischen Auswertung biologischer Bestimmungen, *Arch. f. Gynäk.* **188:** 497–538 (July) 1957.

URINARY EXCRETION OF INTERSTITIAL-CELL STIMULATING HORMONE BY NORMAL MALES AND FEMALES OF VARIOUS AGES*

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ABSTRACT

ICSH activity in the urine of normal males and females of various ages has been determined semi-quantitatively by means of an assay which depends upon the repair of ventral prostatic atrophy in the hypophysectomized immature male rat. The testicular weights of the animals were also recorded as an index of FSH activity. The findings were as follows: (a) In 12 pre-pubertal children, urinary ICSH activity was barely detectable in 3 instances. (b) In 5 adult females, ICSH activity was detectable in mid-cycle, and in 3 of the 5 subjects it was detectable during the follicular and luteal phases as well. There was no appreciable difference between the excretion rates of ICSH during the follicular and luteal phases. However, during mid-cycle a twofold to fourfold increase in ICSH activity occurred. (c) In 17 adult males, ICSH activity was detectable in 16 instances. The excretion of ICSH in hospital patients appeared to be lower than in healthy volunteers. Although the results were variable, it appeared that males excreted appreciably more ICSH than adult females, except when the latter were in the mid-cycle peak phase of the menstrual cycle. (d) In 10 postmenopausal women, the rate of excretion of ICSH was higher than that observed in any other age group of either sex. (e) The increases in testicular and ventral prostatic weights were produced by the same dose of urine concentrate in most instances, suggesting a rough equivalence between the amounts of FSH and ICSH excreted.

PHYSIOLOGIC studies indicate that pituitary interstitial-cell stimulating hormone (ICSH) activity as well as follicle stimulating hormone (FSH) activity is detectable in the urine of adult males (1, 2), adult females (2, 3), and postmenopausal females (4–7). However, little information is

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available concerning the relative amounts of ICSH excreted according to age groups. The purpose of this communication is to present a series of semi-quantitative estimates of ICSH activity in the urine of normal males and females. The data have been obtained by a method (8) which depends upon the repair of ventral prostatic atrophy in the hypophysectomized immature male rat.[1]

METHOD

Urine, collected in 24-hour specimens without the use of a preservative, was concentrated by kaolin adsorption and assayed in hypophysectomized immature male rats. A method previously described (3) was employed, with the single modification that injection of the animals was begun on the third instead of the fifth day after hypophysectomy. The results were rendered semi-quantitative by the use of serial dilutions. A dose interval with a factor of 2 was employed, and that dose yielding a positive response immediately followed by a dose yielding a negative response was taken as the assay result. In most instances, a minimum of 3 rats was employed to test each dose level, pooled specimens being utilized whenever necessary to secure sufficient material for testing. The dosage of urine concentrate to be injected was calculated in terms of square-meter hours in order to compensate for the differences in body surface area between adults and chil-

[1] The specificity of the method has been questioned on the grounds that: 1) in reality, it measures FSH+ICSH, either because of synergism between the two hormones or because FSH and ICSH exist as a complex rather than as separate entities; and 2) it measures ICSH+prolactin.

The evidence which indicates that pituitary ICSH is independent of pituitary FSH in its action upon the Leydig cells has been summarized in an earlier paper (3). The argument (9) that because human chorionic gonadotropin (HCG) is potentiated by FSH in its action upon the interstitial cells, ICSH may be similarly potentiated, is difficult to accept. HCG differs from ICSH not only in its chemical properties but in its biologic effects—for example, upon the accumulation of testicular cholesterol ester (10).

The hypothesis that the pituitary secretes a single gonadotropin and that pituitary FSH and ICSH are chemical artefacts resulting from the cleavage of a mother molecule was apparently first propounded by Saunders *et al.* (11), and has been advanced by a growing number of investigators in recent years. This hypothesis derives its principal support from negative evidence, *i.e.*, from the fact that a parallel separation of gonadotropic hormones in body fluids has not yet been achieved by chemical means. However, as Albert (12) points out in a critical review, the biologic characterization of urinary gonadotropin overwhelmingly favors the existence of at least 2 hormones. Because of the lack of positive evidence in favor of the monistic hypothesis and the existence of a growing body of data supporting the dualistic view (13–16), the authors prefer to interpret their data in terms of the original nomenclature, which implies that ICSH exists as an entity apart from FSH.

The ability of pituitary prolactin preparations to potentiate the action of androgen upon the rat prostate (17, 18) has given rise to the objection (19) that the method, in fact, measures ICSH+prolactin. However, recent investigators employing non-"booster" methods have been unable to detect the presence of prolactin in human urine, even in that of lactating women (20). It seems unlikely, therefore, that prolactin potentiation of significant degree occurs in urinary assays.

dren. The surface area of adults was assumed to be 1.5 square meters, whereas that of children was computed from the child's height and weight.

For purposes of statistical treatment the data were converted to logarithmic form in order to compensate for the increased variation which accompanies increasing weight, and the skewness of "three dimensional" data (*i.e.*, weights, which partake of volume). The rat unit is defined as the least quantity of ICSH which will induce a 33 per cent increase in the weight of the ventral prostate of a 50-gram hypophysectomized male rat. As the geometric mean of the ventral prostatic weights of 339 control animals accumulated over a period of seven years was found to be 7.2 mg. (in logarithms, 0.86 ± 0.11), a geometric mean weight of 9.6 mg. based on data from 3 rats differs significantly from 7.2 mg. ($P = 0.025$). The mean prostatic weight possessing this degree of significance has been adjusted slightly from year to year, in accordance with the prostatic weights of the control animals during the year in which the particular assays were performed.

The weights of the rat testes were also recorded as an index of the amount of FSH activity present. Although the greater part of the increase in testicular weight was believed to result from tubular growth stimulated by FSH, some portion must have reflected ICSH-induced hypertrophy of the Leydig cells and enlargement of the tubules in response to androgen secreted by the interstitial tissue. In the absence of data which would permit appropriate correction for the ICSH content of an unfractionated gonadotropin, such as a urine concentrate, no attempt was made to express the testicular response in terms of rat units of FSH activity. However, if a given dilution of urinary concentrate which yielded a statistically significant increase in ventral prostatic weight concomitantly yielded a statistically significant increase in testicular weight, that fact is indicated in the tables. As the geometric mean of the testicular weights of 336 control animals was found to be 148.6 mg. (in logarithms, 2.172 ± 0.062), a geometric mean weight of 175.0 mg. based on data from 3 rats possesses the same significance ($P = 0.025$) as that elected for the prostatic weight.

RESULTS

A. *Children*

In Table 1 are summarized the results obtained in 6 male and 6 female pediatric patients, who were either admitted to the hospital for study or were convalescent from illnesses not related to the genital tract. None of these children exhibited signs of sexual maturation.

ICSH activity was detectable in the urine of only 3 of the 12 patients. In 2 of the 3 a concomitant increase in testicular weight in the test rats suggested that FSH activity was also present. No excretion data obtained by the same technique are available for comparison.

B. *Adult females*

Five healthy volunteers, all of whom had a normal menstrual history and normal findings on general physical and pelvic examination, served as subjects for this portion of the study. The urine was collected in serial 24-hour specimens throughout the cycle and the basal body temperature was recorded daily. The cycles are presumed to have been ovulatory in character, inasmuch as a pronounced thermal shift occurred in every instance.

TABLE 1. URINARY EXCRETION OF ICSH BY PEDIATRIC PATIENTS

Subject	Disease	Age (yrs.)	Excretion of ICSH/24 hrs. (tested at 2.7 R.U.)
	A. *Males*		
W.S.	Rheumatoid arthritis	6	+*
J.J.	Thermal burns	8½	−
P.S.	Poliomyelitis	8½	−
R.R.	Poliomyelitis	8½	−
R.C.	Study	8½	+
J.G.	Subarachnoid hemorrhage	9	−*
	B. *Females*		
A.S.	Hemiparesis and hemiatrophy	4	−
M.D.	Rheumatic fever	5	−
C.G.	Rheumatic fever	5	−*
J.B.	Adenitis	7	+*
P.M.	Multiple congenital deformities	8½	−
N.S.	Rheumatoid arthritis	11	−*

* Significant increment in weight of testes.

A major objective of the study was to obtain an estimate of the excretion rate of ICSH in mid-cycle, preliminary studies having shown that a peak in excretion occurs at this time. Inasmuch as the cycles varied somewhat in length, a pooling scheme based entirely on the days of the cycle seemed likely to dilute the urine on the peak days with urine from the days immediately preceding or following. Therefore, single-dose assays for the presence or absence of 4 rat units were first performed on a six-hour aliquot of each specimen in order to delimit the period of maximal excretion. In all 5 women, a peak of ICSH excretion was found to extend over a period of two to four days which were closely related in time to the shift in basal body temperature. Pools were next prepared which represented: (a) the mid-cycle days of maximal excretion, (b) the days preceding the ICSH peak (the follicular phase of the cycle), and (c) the days following the ICSH peak (the luteal phase of the cycle). Serially diluted urines were then assayed as in the other portions of the study.

In Table 2 are summarized the excretion rates of ICSH during the different phases of the menstrual cycle. The presence of ICSH activity could be demonstrated during all phases of the cycle in 3 subjects; during only mid-cycle and the follicular phase in 1 (P.W.); and during only mid-cycle in 1 (J.P.). There was no appreciable difference between the excretion rates during the follicular and luteal phases. However, a twofold to fourfold increase in ICSH excretion occurred during mid-cycle.

These data agree with those of Loraine and Brown (21), who found no

TABLE 2. URINARY EXCRETION OF ICSH BY ADULT FEMALES DURING PHASES OF THE MENSTRUAL CYCLE (RAT UNITS/24 HRS.)

Subject	Age (yrs.)	Follicular phase	Mid-cycle	Luteal phase
J.P.	22	—	4*	—
P.W.	22	4*	8*	—
M.W.	22	4*	8	4*
S.C.	26	4*	16	4*
A.B.	27	4*	16*	4*

* Significant increment in weight of testes.

significant difference between the potency of pooled follicular and luteal phase urines, whether ascertained by the rat ventral prostate or mouse uterine weight methods. The data are also in accord with those of Buchholz (22), who determined the ICSH activity of a series of pooled 48-hour urine specimens collected by 17 normal women throughout the menstrual cycle. The ICSH potency of the pools consisting of urines excreted during the follicular and luteal phases did not differ significantly from that of a standard prepared from aliquots of all the specimens collected. However, pools of urine collected on days 13 plus 14 and on days 15 plus 16 of the cycle exhibited potencies which were, respectively, 1.64 and 1.83 times that of the reference standard. The slightly higher potency of our mid-cycle pools as compared with Buchholz' may be due to the difference in the pooling techniques employed.

C. *Adult males*

In Table 3 are summarized the results obtained in 4 hospital patients who were free from diseases of the reproductive tract and in 13 healthy volunteer subjects, several of whom had children. The low levels of ICSH activity in the few hospital patients studied, as compared with the healthy volunteers, are noteworthy. Although an acute increase in the excretion of pituitary gonadotropin was observed by Sohval *et al.* (23) in 6 of 18 patients subjected to operative stress, a decrease in gonadotropin excretion is known to be a characteristic feature of chronic disease (12).

Extreme variability characterized the ICSH excretion levels of the healthy volunteers. In 8 of the 13 subjects the levels were appreciably higher than those observed in adult women during any but the mid-cycle peak phase of the menstrual cycle; in 3 of the 8 the levels were so high as to overlap the lower range for postmenopausal women. No attempt was made to derive a composite figure. However, the results suggest that ICSH activity is greater in healthy men than in women. The same conclusion has been reached by Loraine and Brown (21), who found pooled urine of male

TABLE 3. URINARY EXCRETION OF ICSH BY ADULT MALES

Subject	Disease	Age (yrs.)	ICSH (rat units/24 hrs.)
	A. *Hospital patients*		
N.S.	Rheumatoid arthritis	31	2.7
F.G.	Gout	46	2.7*
E.A.	Rheumatoid arthritis	48	2*
O.T.	Pancreatitis	83	8*
	B. *Healthy volunteer subjects*		
J.C.	—	24	—
R.D.	—	25	12
M.E.	—	25	16
A.G.	—	25	4
Q.S.	—	26	12*
H.U.	—	26	24*
W.K.	—	27	24
C.H.	—	29	4*
B.J.	—	29	2.7*
G.T.	—	29	16*
R.R.	—	30	24*
F.B.	—	32	4*
R.P.	—	46	8*

* Significant increment in weight of testes.

subjects to be approximately four times more active than pooled follicular and luteal phase urine of female subjects.

D. *Postmenopausal females*

In Table 4 are summarized the results obtained in 10 volunteer subjects, all of whom had experienced a spontaneous menopause. A few of the women suffered from minor complicating diseases, such as osteoarthritis, but all were in essentially good health. The level of ICSH activity proved to be higher in this group than in any other studied, although the lower values tended to overlap those obtained in a few of the healthy adult males. Loraine and Brown (21) found pooled menopausal urine to be approximately five times more active than pooled male urine.

E. *Comparison of testicular and prostatic responses*

In Table 5 are summarized the concomitant testicular responses of the test rats to the highest dilutions of urinary concentrate which yielded a statistically significant increase in ventral prostatic weight or, when uniformly negative prostatic responses were obtained, to the highest dilution

TABLE 4. URINARY EXCRETION OF ICSH BY POSTMENOPAUSAL FEMALES

Subject	Age (yrs.)	ICSH (rat units/24 hrs.)
I.H.	50	48*
M.T.	52	96
E.C.	53	48*
B.B.	54	48*
Br.B.	57	96*
E.T.	60	24*
M.P.	65	48*
L.B.	65	96
C.M.	70	96*
L.S.	73	24*

* Significant increment in weight of testes.

tested. In the majority of instances (31 of the 44 yielding a positive response for either or both target organs) significant increases in testicular and prostatic weights occurred concomitantly, suggesting a rough equivalence between the amounts of FSH and ICSH excreted. However, the difficulties attending the interpretation of data obtained from unfractionated

TABLE 5. RESPONSE OF THE TARGET ORGANS TO THE HIGHEST DILUTION OF URINE CONCENTRATE YIELDING A SIGNIFICANT INCREASE IN RAT VENTRAL PROSTATIC WEIGHT, OR (WHEN UNIFORMLY NEGATIVE PROSTATIC RESPONSES WERE OBTAINED) TO THE HIGHEST DILUTION TESTED

	Signif. incr. in wts. of both prostate and testis	Signif. incr. in wt. of prostate but not of testis	Signif. incr. in wt. of testis but not of prostate	Insignif. incr. in wts. of both prostate and testis
Children				
male	1	1	1	3
female	1	0	2	3
Adult males				
patients	3	1	0	0
volunteers	8	4	0	1
Adult females				
follicular phase	4	0	0	1
mid-cycle	3	2	0	0
luteal phase	3	0	0	2
Postmenopausal females	8	2	0	0
Total	*31*	*10*	*3*	*10*

extracts are formidable. Utilizing interstitial-cell repair (a less sensitive indicator than ventral prostatic enlargement) as the criterion for the unit response to ICSH, Bahn *et al.* concluded that there is less ICSH than FSH activity in the urine of both adult males (24) and females (25).

How much significance, if any, should be attached to the 13 instances in which concomitant increases in testicular and prostatic weights of the test rats did not occur is problematical. The 10 instances in which the response to injection of urinary extract was prostatic enlargement without testicular enlargement, were distributed haphazardly among all categories of subjects. On the other hand, the 3 instances of response characterized by testicular enlargement without prostatic enlargement were confined to urines of subjects in the pre-pubertal age group. It is tempting to interpret the latter finding as indicating that the secretion of FSH precedes that of ICSH in childhood. Earlier investigators have reached such a conclusion from the results of urinary (26–28) and pituitary (29–31) assays. Moreover, histologic evidence in harmony with such an interpretation exists in the dichotomy which obtains in the normal pubertal testis (mature or maturing tubules associated with an interstitium containing few if any mature Leydig cells) (32).

COMMENT

The relative amounts of ICSH excreted by subjects in the various age groups, as determined in this study, agree fairly well with the results reported by investigators who measured gonadotropin excretion by means of the mouse uterine weight method. Dekanski (33), for example, found that in order to increase the weight of the mouse uterus by 100 to 150 per cent over that of the controls, the equivalent of 3.2 to 3.9 cc. of urine was required for postmenopausal urine, five times as much for male urine (18.3 cc. or less), and about twenty times as much for normal female urine (62.6 cc. or less). Buchholz (22) did not find any significant qualitative or quantitative differences between the gonadotropin excretion patterns during the normal menstrual cycle, whether obtained by the rat prostate weight or mouse uterine weight methods. In his study, pools of urine collected on days 13 plus 14 and on days 15 plus 16 exhibited potencies which were, respectively, 2.36 and 2.44 times that of the reference standard when measured by the mouse uterine weight method. A marked discrepancy, on the other hand, obtains with respect to potencies during the different phases of the menstrual cycle as determined by the rat ovarian (12) and ventral prostatic weight methods.

These various findings are susceptible to a number of interpretations. It may be, for instance, that FSH and ICSH vary independently during the different phases of the menstrual cycle and that the ovarian weight method

measures FSH+ICSH, whereas the uterine weight response, like the prostatic response, is largely dependent upon the ICSH activity of the extracts.

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REFERENCES

1. EVANS, H. M., and GORBMAN, A.: Urinary gonadotrophins in normal men, *Proc. Soc. Exper. Biol. & Med.* **49:** 674, 1942.
2. HELLER, C.: Comment on paper of Greep, R. O., and Jones, I. C.: Steroid control of pituitary function, *Recent Prog. Hormone Res.* **5:** 197, 1950.
3. McARTHUR, J. W.: The identification of pituitary interstitial cell stimulating hormone in human urine, *Endocrinology* **50:** 304, 1952.
4. TYNDALE, H. H.; LEVIN, L., and SMITH, P. E.: Responses of normal and hypophysectomized immature rats to menopause urine injections, *Am. J. Physiol.* **124:** 174, 1938.
5. TYNDALE, H. H.: quoted by Engle, E. T., *in* Sex and Internal Secretions, ed. 2, E. A. Allen, Editor. Baltimore, Williams and Wilkins Co., 1939, chapter 18.
6. GREEP, R. O., and JONES, I. C.: Steroid control of pituitary function, *Recent Progr. Hormone Res.* **5:** 197, 1950.
7. McARTHUR, J. W.: The bioassay of pituitary interstitial cell stimulating hormone (ICSH) in human urine: preliminary report, (abstract) *J. Clin. Endocrinol. & Metab.* **12:** 914, 1952.
8. GREEP, R. O.; VAN DYKE, H. B., and CHOW, B. F.: Use of anterior lobe of prostate gland in the assay of metakentrin, *Proc. Soc. Exper. Biol. & Med.* **46:** 644, 1941.
9. BROWN, P. S.: Experimental examination of an assay for urinary follicle stimulating hormone, *J. Endocrinol.* (British) **14:** 257, 1956.
10. TEPPERMAN, J., and TEPPERMAN, H. M.: Effects of purified gonadotrophins on the cholesterol content of the testes of immature rats, *Endocrinology* **50:** 162, 1952.
11. SAUNDERS, F. J., and COLE, H. H.: On the reliability of present methods for characterizing two gonadotropic hormones, follicle-stimulator and luteinizer, *Endocrinology* **23:** 302, 1938.
12. ALBERT, A.: Human urinary gonadotropin, *Recent Progr. Hormone Res.* **12:** 227, 1956.
13. BROWN, P. S.: Fractionation of human urinary gonadotrophins, *Nature* **178:** 315, 1956.
14. BROWN, P. S.: Follicle stimulating and interstitial cell stimulating hormones in the urine of women with amenorrhea, *J. Endocrinol.* (British) **14:** 129, 1956.
15. WALTER, K.: Qualitative differences in gonadotropic extracts from urine of postmenopausal women, *J. Endocrinol.* (British) **15:**119, 1957.
16. HAMBURGER, C., and JOHNSEN, S. G.: Studies on urinary gonadotrophins. III. Qualitative differences between urinary gonadotrophins of young males and of postmenopausal women, *Acta endocrinol.* **26:** 1, 1957.
17. GRAYHACK, J. T.; BUNCE, P. L.; KEARNS, J. W., and SCOTT, W. W.: Influence of the pituitary on prostatic response to androgen in the rat, *Bull. Johns Hopkins Hosp.* **96:** 154, 1955.

18. SEGALOFF, A.; STEELMAN, S. L., and FLORES, A.: Prolactin as a factor in the ventral prostate assay for luteinizing hormone, *Endocrinology* **59**: 233, 1956.
19. SEGALOFF, A.; GORDON, D.; CARABASI, R. A.; HORWITT, B. N.; SCHLOSSER, J. V., and MURISON, P. J.: Hormonal therapy in cancer of the breast. VII. Effect of conjugated estrogens (equine) on clinical course and hormonal excretion, *Cancer* **7**: 758, 1954.
20. BAHN, R. C., and BATES, R. W.: Histologic criteria for detection of prolactin: lack of prolactin in blood and urine of human subjects, *J. Clin. Endocrinol. & Metab.* **16**: 1337, 1956.
21. LORAINE, J. A., and BROWN, J. B.: Further observations on the estimation of urinary gonadotropins in non-pregnant human subjects, *J. Clin. Endocrinol. & Metab.* **16**: 1180, 1956.
22. BUCHHOLZ, R.: Untersuchungen über die Ausscheidungsverhältnisse der gonadotropen Hypophysenhormone FSH und ICSH im mensuellen Cyclus, *Ztschr. ges. exper. Med.* **128**: 219, 1957.
23. SOHVAL, A. R.; WEINER, I., and SOFFER, L. J.: The effect of surgical procedures on urinary gonadotropin excretion, *J. Clin. Endocrinol. & Metab.* **12**: 1053, 1952.
24. BAHN, R. C.; LORENZ, N.; BENNETT, W. A., and ALBERT, A.: Gonadotropins of the pituitary gland and urine of the adult human male, *Proc. Soc. Exper. Biol. & Med.* **82**: 777, 1953.
25. BAHN, R. C.; LORENZ, N.; BENNETT, W. A., and ALBERT, A.: Gonadotropins of the pituitary gland and the urine of the adult human female, *Endocrinology* **52**: 135, 1953.
26. PHILIPP, E.: Sexualhormone, Placenta und Neugeborenes. Experimentelle Studie, *Zentralbl. Gynäk.* **53**: 2386, 1929.
27. NEUMANN, H. O., and PETER, F.: Die Hormonausscheidungen im Kindesalter, *Ztschr. Kinderh.* **52**: 24, 1931.
28. SOEKEN, G.: Zur physiologie der Pubertät. Die Ausscheidung des Hypophysenvorderlappenhormon-A im Urin, *Ztschr. Kinderh.* **53**: 339, 1932.
29. WIRZ, P.: Hypophysenvorderlappenhormone und Amenorrhöe, *Ztschr. Geburtsh. u. Gynäk.* **104**: 293, 1933.
30. SAXTON, J., and LOEB, L.: Thyroid stimulating and gonadotropic hormones of the human anterior pituitary gland at different ages and in pregnant and lactating women, *Anat. Rec.* **69**: 261, 1937.
31. BAHN, R. C.; LORENZ, N.; BENNETT, W. A., and ALBERT, A.: Gonadotropins of the pituitary gland during infancy and early childhood, *Endocrinology* **52**: 605, 1953.
32. ALBERT, A.; UNDERDAHL, L. O.; GREENE, L. F., and LORENZ, N.: Male hypogonadism. 1. The normal testis, *Proc. Staff Meet., Mayo Clin.* **28**: 409, 1953.
33. DEKANSKI, J.: The kaolin-adsorption method for the quantitative assay of urinary gonadotrophins, *Brit. J. Exper. Path.* **30**: 272, 1949.

CEREBROSPINAL FLUID PROTEINS IN MYXEDEMA, WITH SPECIAL REFERENCE TO ELECTROPHORETIC PARTITION*

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ABSTRACT

The dynamics and constituents of the cerebrospinal fluid were studied in 27 consecutive patients with myxedema. The only significant abnormality noted was elevation of the level of total protein in 25 patients. Electrophoretic partition revealed a parallel increase of the gamma globulin fraction of the protein in both cerebrospinal fluid and serum. No correlation was found between the degree of elevation of the cerebrospinal fluid protein level and the clinical status of the patient.

ALTHOUGH elevation of the cerebrospinal fluid protein level in myxedema was reported by Thompson *et al.* in 1929 (1), it has been our experience that it is common for this finding to be misinterpreted as evidence of organic brain disease apart from myxedema. It is the purpose of this paper to: 1) re-emphasize the frequency with which elevation of the cerebrospinal fluid protein level occurs in myxedema, 2) report the results of analysis of the cerebrospinal fluid proteins by electrophoretic partition, and 3) correlate the clinical findings in myxedema with the spinal fluid findings.

SUBJECTS AND METHODS

Twenty-nine patients with myxedema, 27 of whom were seen consecutively and all but 1 (No. 26) of whom were untreated, were subjects of this study. In each, the 24-hour uptake of radioactive iodine by the thyroid gland was less than 6 per cent. Cerebrospinal fluid was obtained for analysis by lumbar puncture, while the patients were in the nonfasting state. The total protein concentration of the fluid was determined by the sulfosalicylic-acid method (2), for which the normal range in this laboratory is between 25 and 50 mg. per 100 cc. For colloidal gold curves, the method of Lange was used (3). Blood samples were obtained simultaneously with the spinal fluid, and analyzed for total protein, glucose, and chloride concentrations.

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In 9 of the patients, paper electrophoresis of the cerebrospinal fluid proteins was carried out by the following method: A 10-cc. sample of cerebrospinal fluid was withdrawn and immediately centrifuged. The supernatant fluid was frozen quickly and stored at −5° C. until analysis. After thawing, the entire volume was dialyzed against 20 per cent polyvinylpyrrolidone[1] (PVP) for sixteen to twenty-four hours, to a volume of approximately 0.20 cc. To this was added 0.25 cc. of 0.1 *M* veronal buffer (pH 8.6). The solution was thoroughly mixed and a 40-μg. portion was applied to filter paper. Electrophoresis was performed by the method of Durrum (4) for sixteen hours. The filter-paper strip was stained with bromophenol blue and the electrophoretic partition determined by use of a Spinco Model "R" Analytrol. In 4 of the 9 patients, paper electrophoresis of serum was performed simultaneously.

For comparison, the electrophoretic partitions of the proteins of both serum and cerebrospinal fluid were determined in 6 nonmyxedematous patients. These patients, 5 men and 1 woman, between the ages of 48 and 86, were undergoing elective surgery (for hernia, hypertension, femoral fracture, or arterial insufficiency) and cerebrospinal fluid was obtained prior to the induction of spinal anesthesia.

In 12 of the patients with myxedema, multichannel electroencephalograms were obtained for correlation with spinal fluid findings.

RESULTS

In Table 1 are summarized the clinical data and the levels of total protein, glucose, and chloride in the cerebrospinal fluid and blood of 27 patients with myxedema. The cerebrospinal fluid pressure was between 200 and 270 mm. of water in 5 patients, and between 180 and 200 mm. of water in 5 others. The fluid was crystal clear and contained the normal numbers of cells. None of the fluids presented serologic evidence of syphilis, but the colloidal gold curves frequently showed a slight discoloration in the midzone; a value above 2 was not observed. The mean spinal fluid glucose concentration was 70 per cent of that in the blood (range, 40–90 per cent), and the mean chloride concentration was 117 per cent of that in the serum (range, 103–129 per cent).

Abnormal elevation of the level of spinal fluid protein was observed in 25 patients (mean value, 98 mg. per 100 cc.). A concentration greater than 100 mg. per 100 cc. was found in 12 patients. The highest protein concentration observed, after exclusion of patients with complicating illnesses, was 168 mg. per 100 cc. In Patient No. 22, the protein concentration was 62 mg. per 100 cc.; previously, when she had been hyperthyroid, the concentration had been 26 mg. per 100 cc. The Pandy reaction was positive in all fluids with an elevated protein content. The concentration of total protein in serum was normal in all but 1 patient (No. 25).

The electrophoretic partition of cerebrospinal fluid protein in 9 patients with myxedema is presented in Table 2. A comparison of these findings with the electrophoretic pattern of the cerebrospinal fluid protein in 6

[1] Obtained from Abbott Laboratories, North Chicago, Illinois.

TABLE 1. LEVELS OF TOTAL PROTEIN, GLUCOSE AND CHLORIDE IN CEREBROSPINAL FLUID AND BLOOD OF 27 PATIENTS WITH MYXEDEMA

Pt. No.	Age & sex	Type of myxedema	BMR (%)	Total serum cholesterol (mg./100 cc.)	Cerebrospinal fluid			Blood			Remarks
					Protein (mg./100 cc.)	Glucose (mg./100 cc.)	Chloride (mEq./100 cc.)	Protein (Gm./100 cc.)	Glucose (Gm./100 cc.)	Chloride (mEq./100 cc.)	
1	58 M	Postop. & post I^{131}	−38	387	340	87	120	8.1	107		Post-radical neck dissection for carcinoma of thyroid. Carcinoma of prostate
2	54 M	Post. I^{131}	−25	235	168	80	120	7.5	99	104	
3	70 M	Primary	−20	262	148	110	130	7.5	120	107	
4	68 F	Postop.	−45	660	134	92	116	7.7	193	98	Rheumatoid arthritis
5	35 F	Primary	−45	350	122	75	119	7.3	187	100	
6	60 F	Postop.	−34	465	110	92	119	7.0	110		Recent cerebral thrombosis. Blood Kahn and Wassermann reactions positive
7	66 F	Postop.		500	104	70	115	6.1	90	89	Paranoid psychosis. Rheumatoid arthritis
8	68 F	Primary	−34	340	104	75	119	7.0	106	104	
9	38 F	Primary	−30	277	100	82	126	7.9	117	100	
10	71 F	Primary	−32	316	100	41	116	7.3	119	104	
11	56 M	Primary	−21	277	100	72	119	6.0			
12	68 M	Primary	− 2	222	100	64	115	7.5	114		Myelocytic leukemia
13	73 F	Primary	−35	330	97	85	124	6.8	105	107	
14	58 F	Primary	−38	285	94	58	122	6.2	120	109	
15	62 F	Postop.	−24	400	94	90	110	7.2	150		Paranoid psychosis
16	58 F	Primary	−43	255	94	67	111	6.1	87	94	Pericardial effusion. One episode of psychosis
17	61 F	Primary	− 8	362	82	60	111	7.0		107	
18	76 F	Primary		250	78	98	120	7.0	117	100	
19	51 F	Postop.	−21	415	68	107	122	7.9	130	100	Localized pretibial myxedema
20	74 M	Primary	−37	240	68	80	120	7.2			
21	76 F	Primary	−28	375	62	82	111	7.2	120	100	
22	49 F	Postop.	−26	200	62	80	126	8.0	114	109	Spinal fluid protein conc. 26 mg./100 cc. when patient hyperthyroid
23	46 F	Post. I^{131}	−33	438	59	70	116	8.0	117	104	
24	61 F	Primary	−29	168	52	64	120	7.3			
25	78 F	Primary	−37	307	52	92	115	5.3	120	91	Autopsy: severe primary myxedema
26	73 F	Primary	−29	262	32	75	82	7.4	74	112	Ascites. Died in state of adrenal insufficiency
27	53 F	Postop. & post. I^{131}	−41	320	29	68	115	7.6	100	98	

TABLE 2. RESULTS OF ELECTROPHORETIC PARTITION OF PROTEIN OF CEREBROSPINAL FLUID AND SERUM IN 9 PATIENTS WITH MYXEDEMA

Pt. No.	Specimen	Total protein (Gm./100 cc.)	Partition of protein (relative %)				
			Albumin	Alpha-1 globulin	Alpha-2 globulin	Beta globulin	Gamma globulin
4	CSF	0.134	36	8	11	19	26
12	CSF	0.100	55	6	6	10	23
14	CSF	0.094	36	6	15	21	22
15	CSF	0.070	41	7	8	19	25
22	CSF	0.062	55	4	8	17	16
24	CSF	0.052	43	6	6	15	30
	Serum	6.2	43	4	7	10	36
26	CSF	0.032	32	7	10	28	23
	Serum	7.4	39	4	10	14	33
28	CSF*	0.032	36	6	6	20	30
	Serum	7.1	41	6	8	11	34
29	CSF	0.021	25	8	10	31	26
	Serum	6.3	46	6	8	15	25

* Pre-albumin fraction, 2%.

hospitalized control patients is presented in Table 3. The patients with myxedema showed a significantly greater concentration of total protein in the cerebrospinal fluid and a significantly greater concentration of gamma globulin than did the controls. The concentration of gamma globulin in serum was also significantly increased in myxedema (Table 3). The other fractions of both cerebrospinal fluid and serum proteins were normal. In both the myxedema and control groups, the pattern of proteins in the cerebrospinal fluid was not significantly different from that in the serum. No pre-albumin fraction of spinal fluid proteins (5) was observed in myxedema, and this fraction was found in only 1 control patient.

The electroencephalograms obtained in 12 patients, all with an elevated concentration of cerebrospinal fluid protein, were characterized primarily by low-voltage recordings. When this was severe, the alpha waves could not be discerned and frequency patterns were difficult to ascertain. Another feature that has been noted in myxedema—absence of reaction to light stimulation (6)—was not sought for in this study.

TABLE 3. MEAN AND RANGE OF ELECTROPHORETIC PROTEIN FRACTIONS IN CEREBROSPINAL FLUID AND SERUM IN MYXEDEMA AND CONTROL PATIENTS

	Group	No. of pts.	Total protein (Gm./100 cc.)	Partition of protein (relative %)				
				Albumin	Alpha-1 globulin	Alpha-2 globulin	Beta globulin	Gamma globulin
Cerebrospinal fluid	Myxedema	9	0.066 (.021–.134)	40 (25–55)	6 (4–8)	9 (6–15)	20 (10–31)	25 (16–30)
	Controls†	6	0.031 (.017–.045)	45 (40–56)	6 (3–12)	9 (7–12)	21 (15–28)	18 (12–22)
	Difference; "t" test		2.77*	1.43	0.20	0.04	0.36	3.16**
Serum	Myxedema	8	6.8 (6.2–7.4)	40 (35–47)	6 (4–9)	10 (7–16)	15 (10–20)	29 (22–36)
	Controls	6	6.6 (5.6–7.1)	42 (32–49)	5 (4–8)	13 (9–17)	18 (16–22)	22 (12–26)
	Difference; "t" test		1.30	0.57	1.09	1.94	2.06	3.08**

* Significant at 5% level.
† Pre-albumin fraction, 1% (range, 0–2%).
** Significant at 1% level.

No correlation could be found between the degree of elevation of the cerebrospinal fluid protein and the clinical status of the patient. Three patients were psychotic or had a history of psychosis; in all 3 the protein concentration in the cerebrospinal fluid was high. No psychic abnormalities, however, were present in the other 24 patients. The clinical severity of the myxedema did not parallel the degree of elevation of the level of cerebrospinal fluid protein. Normal spinal fluid protein levels were observed in patients with severe, long standing, untreated myxedema. Low-voltage electroencephalograms were found in patients with and without psychosis accompanying myxedema.

The presence of myxedema did not affect the development of spinal fluid changes characteristic of other diseases. For instance, Patient No. 6 had a recent cerebral thrombosis, Patient No. 1 had vertebral metastasis from prostatic carcinoma, and Patient No. 26 had an addisonian-like crisis following initiation of thyroid therapy; and all 3 exhibited spinal fluid abnormalities consistent with these conditions.

DISCUSSION

In the study by Thompson *et al.* (1), elevation of the cerebrospinal fluid protein concentration was found in 13 of 17 patients with myxedema. A high protein content was also demonstrated in fluid obtained by cisternal puncture. The protein levels returned to normal when the euthyroid state was achieved by thyroid therapy. In the present study, elevation of the cerebrospinal fluid protein concentration was found in 25 of 27 consecu-

tive patients, in 12 of whom the concentration was greater than 100 mg. per 100 cc. These findings are in agreement with those of Thompson *et al.* That the protein content of the fluid is related to the thyroid status is strengthened by the findings of Thompson and Alexander (7) that, in hyperthyroidism, there is a low concentration of cerebrospinal fluid protein which increases significantly following successful treatment.

In myxedema, no other significant cerebrospinal fluid abnormalities besides elevation of the protein content have been found. Thompson *et al.* observed that the ratio of the cerebrospinal fluid chloride level to the serum chloride level decreased with thyroid therapy. In the present study, the levels of chloride, glucose, the character of the fluid, the cellularity, the Wassermann reaction, and the colloidal gold curves were normal in uncomplicated myxedema. Lups and Haan (8) noted moderate elevation of the cerebrospinal fluid pressure in myxedema, and in this study a pressure over 180 mm. of water was found in 10 of the 21 patients in whom this determination was made.

Although electrophoretic studies of the cerebrospinal fluid protein in myxedema have not previously been reported, studies of the *serum* protein by this method have shown abnormal patterns. Lewis and McCullagh (9), using free electrophoresis, found diminished albumin and alpha globulin and increased beta globulin; Lamberg and Grasbeck (10), using paper electrophoresis, noted similar changes; and Mahaux and Koïw (11) noted elevation of the gamma globulins.

In the present study, paper electrophoresis of serum proteins revealed that the value for albumin in myxedema was lower than that usually reported as the normal value. However, in the hospital control patients the value for albumin was also lower than normal, and there was no significant difference between the two groups. It should be noted that the majority of patients in both groups were more than 60 years old—an age group in which the serum albumin level is substantially lower than that in young adults (12). In contrast to most of the studies cited, the only significant abnormality of serum protein observed in our group of patients with myxedema was an elevation of the gamma globulin value.

Paper electrophoresis of cerebrospinal fluid proteins likewise revealed an increase in the relative proportion of gamma globulin in myxedematous patients compared to non-myxedematous controls. The other fractions occurred in proportions similar to those found in the hospitalized controls. A pre-albumin fraction is usually found in the majority of spinal fluids ((5) and Bronsky *et al.*, unpublished observations) but in this study it was found in the fluid of only 1 of the 6 control patients. The significance, therefore, of the uniform failure to find a pre-albumin fraction in the spinal fluid of patients with myxedema cannot be evaluated.

The degree of elevation of the gamma globulin fraction was insufficient

to account for the increase in the total protein content of the cerebrospinal fluid. The findings can be explained on the basis of an alteration in the permeability of the blood-cerebrospinal fluid barrier, which allows a generalized increase in all cerebrospinal fluid protein fractions in direct proportion to their occurrence in the serum. This would result, not only in an increased concentration of total protein, but an elevation of the gamma globulin fraction as well.

Thompson *et al.* concluded that myxedema was accompanied by altered permeability of the capillary membranes throughout the body. The conclusion of the present study is in agreement with this hypothesis. The absence of correlation between the clinical status of the patient and cerebrospinal fluid and electroencephalographic findings does not permit conclusions as to the pathogenesis of the change in permeability.

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REFERENCES

1. Thompson, W. O.; Thompson, P. K.; Silveus, E., and Dailey, M. E.: The cerebrospinal fluid in myxedema, *Arch. Int. Med.* **44**: 368–373, 1929.
2. Ayers, J. B.; Dailey, M. E., and Fremont-Smith, F.: Denis-Ayer method for the quantitative estimation of protein in the cerebrospinal fluid, *Arch. Neurol. & Psychiat.* **26**: 1038–1042, 1931.
3. Levine, J. S.: Making gold sol for spinal fluid tests, *Am. J. Syph.* **16**: 103–109, 1932.
4. Jencks, W. P.; Jetton, M. R., and Durrum, E. L.: Paper electrophoresis as a quantitative method: serum proteins, *Biochem. J.* **60**: 205–215, 1955.
5. Fisk, A. A.; Chanutin, A., and Klingman, W. O.: Rapidly migrating electrophoretic component, *Proc. Soc. Exper. Biol. & Med.* **78**: 1–3, 1951.
6. Scheinberg, P.; Stead, E. A., Jr.; Brannon, E. S., and Warren, J. V.: Correlative observations on cerebral metabolism and cardiac output in myxedema, *J. Clin. Invest.* **29**: 1139–1146, 1950.
7. Thompson, W. O., and Alexander, B.: Exophthalmic goiter: the protein content of the cerebrospinal fluid, *Arch. Int. Med.* **45**: 122–124, 1930.
8. Lups, S., and Haan, A.: The Cerebrospinal Fluid. New York, Elsevier Publishing Co., 1954.
9. Lewis, L. A., and McCullagh, E. P.: Electrophoretic analysis of plasma proteins in hyperthyroidism and hypothyroidism, *Am. J. M. Sc.* **208**: 727–735, 1944.
10. Lamberg, B. A., and Grasbeck, R.: The serum protein pattern in disorders of thyroid function, *Acta endocrinol.* **19**: 91–100, 1955.
11. Mahaux, J., and Koïw, E.: Le protëinogramme et le lipidogramme sur papier filtre dans le myxoedème. Action de la thyroxine, *Ann. endocrinol.* **13**: 691–696, 1952.
12. Chesrow, E. J.; Bronsky, D.; Orfei, E.; Dyniewicz, H.; Dubin, A., and Musci, J.: Serum proteins in the aged: means and stability of mucoprotein levels and electrophoretic partitions, *Geriatrics* **13**: 20–24, 1958.

METABOLIC OBSERVATIONS IN A CASE OF THYROTOXICOSIS WITH HYPERCALCEMIA*

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ABSTRACT

Hyperthyroidism characteristically causes excessive urinary and fecal excretion of calcium and, rarely, hypercalcemia. These abnormalities were studied in a 58-year-old thyrotoxic male. The investigation consisted of a 125-day balance study as the patient became euthyroid and a similar 50-day balance study during a recurrence of the thyrotoxicosis. The excessive urinary and fecal loss of calcium bore little relationship to the calcium or caloric content of the diet or to the level of nitrogen balance. A high phosphate diet ameliorated the metabolic abnormality and corrected the hypercalcemia. Successful treatment of the thyrotoxicosis produced similar changes in calcium metabolism. Hypercalcemia was not present during recurrence of the thyrotoxicosis.

The metabolic observations in this case and those previously reported strongly suggest that the excessive urinary and fecal excretion of calcium in thyrotoxicosis is due to a direct effect of thyroid hormone on osseous metabolism. The hypercalcemia is probably secondary to a disorder in the homeostatic mechanism(s) regulating the content of ionized calcium in the extracellular fluid.

THYROID dysfunction at all ages results in abnormalities of osseous metabolism (1, 2). This is reflected in alterations in the metabolism of the skeleton's principal element, calcium. Both clinical and experimental thyrotoxicosis may be associated with an excessive loss of calcium through *both* the gastro-intestinal tract and kidneys. In fact, as stated by Aub *et al.*, "the calcium drain in exophthalmic goiter is even greater than in hyperparathyroidism." At times the loss may be so extreme that gross

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clinically detectable demineralization of the skeleton occurs (3). Demineralization of this type has frequently been called "thyrotoxic osteoporosis" (4–6) but, as will be shown subsequently, this is probably a misnomer.

The exact mechanism of the abnormality in calcium metabolism is still unclear. More difficult to explain is the rare association of hypercalcemia and thyrotoxicosis, as demonstrated by the present case and a few previously reported (7–10). The presence of hypercalcemia may greatly confuse the clinical picture of thyrotoxicosis.

The present metabolic investigation was undertaken to study 1) the effect of various procedures and therapy on the disordered calcium metabolism of thyrotoxicosis, and 2) the mechanisms involved in associated hypercalcemia.

CASE REPORT

L.C., a 56-year-old Negro male retired civil service worker, was admitted September 7, 1956. His chief complaints were weight loss, anorexia, epigastric "soreness," and pruritis.

For approximately a year there had been progressive weight loss. For three to four months this had not been associated with a loss of appetite but, for at least eight months, anorexia had been a prominent symptom, associated with occasional nausea and vomiting. Epigastric "soreness" had been present for approximately one year. Bowel movements had increased to three to four per day during the three months prior to admission. The pruritis was generalized and had increased gradually for a period of three to four months prior to admission. The patient had noted intolerance to heat and progressive weakness of his arms and legs. He volunteered the observation that his already dark pigmentation was becoming even darker. Polyuria and polydipsia had developed at least six months prior to admission. There was no history of excessive ingestion of milk, alkali, or vitamin D.

Physical examination disclosed a chronically ill, undernourished Negro. He was apathetic. The blood pressure was 150 mm. Hg systolic and 80 mm. diastolic, and it remained near these levels throughout his hospital stay. The pulse ranged from 90 to 110. The skin was almost blue-black in color, but there was no abnormal pigmentation of the mucous membranes. Generalized muscular wasting was evident. There was a fine tremor of the outstretched hands, and the palms were warm and moist. There were no abnormal eye signs. The thyroid was approximately twice normal size, somewhat firm, but not nodular. Heart sounds were hyperactive, and loud apical and aortic systolic murmurs were heard. The lungs were normal. There was diffuse generalized abdominal tenderness, but no organs or masses were palpable. The genitalia were normal, as was the distribution of hair. There were no physical abnormalities of the bones or joints. Deep tendon reflexes were hyperactive.

The urinary sediment contained 1 to 3 white and red blood cells per high-power field. Urinary protein excretion was 80 to 100 mg. per twenty-four hours. The specific gravities of random samples of urine varied between 1.010 and 1.018. The blood hemoglobin level ranged between 11 and 12 grams per 100 ml. The following concentrations were found in serum: sodium, potassium, CO_2 and chlorides within normal limits; creatinine, 0.6 to 1.1 mg. per 100 ml.; total protein 7.4 Gm., albumin 4.3 Gm., globulin 3.1 Gm. per 100 ml.; and cholesterol, 165–180 mg. per 100 ml.

The serum levels of calcium and phosphorus prior to transfer to the Metabolic ward were:

Calcium (*mg./100 ml.*)	*Phosphorus* (*mg./100 ml.*)	*Alk. Phosphatase* (*Bod. units/100 ml.*)
12.0	4.0	
13.6	3.9	5.2
12.8	4.3	4.7
11.3	4.0	

Fasting blood sugar concentration and a glucose tolerance curve were normal.

The level of serum protein-bound iodine (PBI) was 17.4 μg. per 100 ml. The thyroidal uptake of I^{131} was 63 per cent in six hours and 66 per cent in twenty-four hours. Basal metabolic rates were +23 and +28 per cent. A radioactive iodine scintigram disclosed a slightly enlarged, diffusely hyperactive thyroid gland. Basal urinary 17-ketosteroid and 17-hydroxycorticoid excretion values were 5.7 and 6.6 mg. per twenty-four hours, respectively (normal, 7.0 to 18.0 for 17-KS, and 1.1 to 10.0 for 17-OH-CS). After an eight-hour infusion of ACTH, these values rose to 9.3 and 11.3 milligrams per twenty-four hours, respectively.

Roentgenograms of the spine, skull, pelvis, long bones and lamina dura disclosed no demineralization, osteitis fibrosa cystica, subperiosteal bone resorption, or soft-tissue calcification. There were no renal calcifications.

METHODS AND PROCEDURES

The metabolic study was divided into two parts. Part I (125 days) encompassed the treatment phase during which the patient returned to the euthyroid state. Part II (50 days) was undertaken during a recurrence of the thyrotoxic state. Each part was divided into five-day collection periods. The patient was studied on a metabolic ward. The calcium, phosphorus and nitrogen of the diet were kept constant during a particular program. Sample diets which duplicated the diet of the patient were prepared at intervals of ten days or whenever there was a dietary change, and analyzed to determine intake of the various constituents. During the first 25 periods of the study (Part I) the diet contained (with the exception of periods 4 and 5) $64.90 \pm$ S.D.[1] 1.33 Gm. of nitrogen, $5140 \pm$ S.D. 139 mg. of calcium, and $5560 \pm$ S.D. 162 mg. of phosphorus per period. During periods 4 and 5, the calcium intake was reduced to 1235 mg. per period (247 mg. per day), but the nitrogen and phosphorus content of the diet was not changed. In periods 11 and 12, the intake of phosphorus was increased by giving, in addition to the standard diet, 120 ml. per day of sodium phosphate solution buffered to neutrality and containing 15.72 ± 0.08 mg. of phosphorus per ml. This was administered three times daily in fractional doses and sipped with meals.

During the last 10 periods of study (Part II), the diet contained 63.90 ± 0.82 Gm. of nitrogen, 5290 ± 89 mg. of calcium, and 5690 ± 76 mg. of phosphorus per period. During periods 5, 6 and 7, additional calcium was given as a solution of calcium acetate containing 32.54 ± 0.14 mg. of calcium per ml. The patient received 50 ml. of this solution in fractional doses three times daily with meals.

Urine was collected over 24-hour periods, refrigerated without preservative, and analyzed daily for phosphorus and nitrogen. Aliquots of the 24-hour collections were

[1] One standard deviation.

saved and pooled in periods of five days. The pooled samples were analyzed for calcium, nitrogen and phosphorus. Stools were separated at intervals of five days and also analyzed for calcium, nitrogen and phosphorus.

The calcium concentrations of the serum and of the calcium acetate solution were determined by the method of Kochakian and Fox (11). The serum and the buffered phosphate solution were analyzed for phosphorus and the feces, urine and diet for calcium, phosphorus and nitrogen, by other previously reported methods (12–14). The analyses were performed on aliquots of diet, urine and feces after they had been ashed in a muffle furnace at a temperature not exceeding 450° C.

RESULTS

Clinical observations

This patient, although presenting many of the characteristic symptcms of thyrotoxicosis, complained of marked anorexia, epigastric "distress," and recurrent nausea and pruritis. The gastro-intestinal complaints, although most unusual in hyperthyroidism, are the commonest symptoms associated with hypercalcemia. They have been prominent in other cases of thyrotoxicosis with hypercalcemia (7–10).

On the metabolic ward prior to specific therapy, symptoms of thyrotoxicosis persisted. Although mild nausea was a frequent complaint, there was no vomiting, and the patient ate everything given. The pruritis continued until the serum calcium levels had returned to normal.

Two weeks after the administration of a therapeutic dose of I^{131} (2.4 millicuries at the start of period 11), there was a decrease in heat intolerance, tremor, and the hyperactivity of the reflexes. Propylthiouracil was started during period 16 in an effort to enhance the rate of return to a euthyroid state. The patient was euthyroid prior to discharge—serum PBI level, 5.8 μg. per 100 ml.

During the ensuing eight weeks, thyrotoxicosis with its associated symptoms recurred. The serum PBI level was 11 μg. per 100 ml., and the thyroidal I^{131} uptake was 42 per cent at six hours and 69 per cent at twenty-four hours. However, at the time of this admission anorexia, nausea, vomiting and pruritis were not present. The symptoms and signs of hyperthyroidism persisted throughout the second balance study (50 days).

Caloric intake, weight, and nitrogen balance (Fig. 1; Table 1)

From periods 1 through 8, while the intake averaged 2200 calories, there was a slight but definite decrease in body weight. When the intake was increased to approximately 2900 calories (period 9), there was a 3-kilogram gain in weight between periods 9 and 13. Increase in weight was progressive subsequent to the administration of propylthiouracil, despite a decrease of approximately 300 calories subsequent to period 22.

A protein intake of 81 Gm. was maintained as constantly as possible

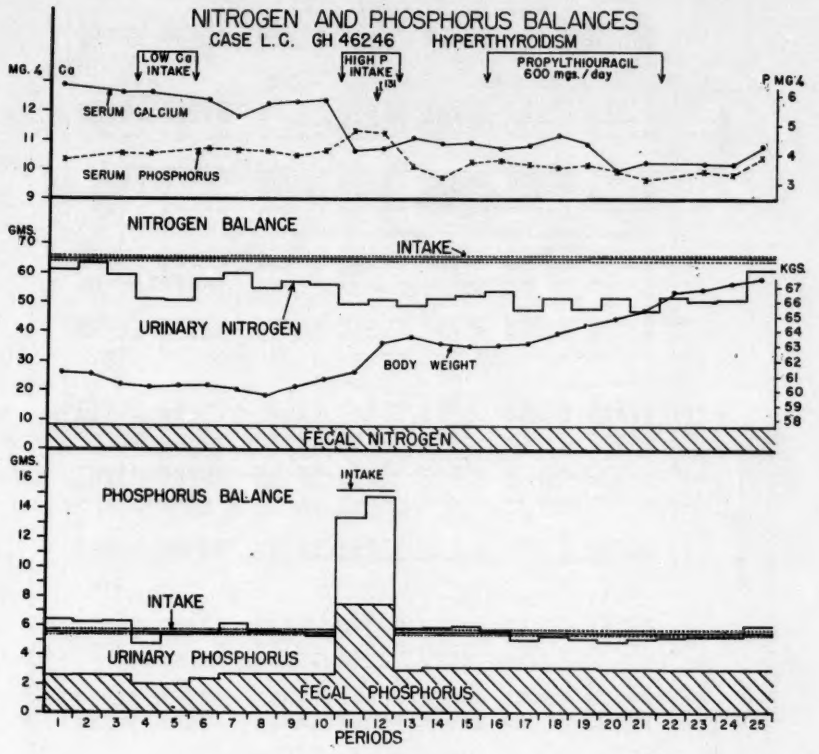

FIGURE 1

throughout the entire study (Parts I and II). Even though there was an early negative caloric balance, nitrogen balance was consistently positive. The maximal positive balance of nitrogen was attained during the period of propylthiouracil administration. The total positive balance of nitrogen (corrected for dermal loss, 0.4 Gm. per day (15)) for the 125 days approximated 236 Gm. If we assume that the protein content of "lean tissue" is about 20 per cent, then the amount of fat-free tissue deposited was 7.4 Kg.—a figure similar to the gain in body weight.

The recurrence of thyrotoxicosis was not associated with any striking loss of weight or of nitrogen (Fig. 2; Table 1).

Calcium and phosphorus balances

Throughout the entire study, with the exception of the brief high and low calcium periods, the patient ingested about 1 Gm. of calcium daily. Bogdonoff, Shock and Nichols (16) have shown that normal American

TABLE 1. DATA AND BALANCE CALCULATIONS FOR PARTS I AND II OF THE STUDY

Period	Procedures	Serum values (mg./100 ml.)		Body wt. (kg.)	Nitrogen (Gm.)				Calcium (mg.)				Phosphorus (mg.)			
		Ca	P		Urine	Feces	Total	Bal.	Urine	Feces	Total	Bal.	Urine	Feces	Total	Bal.
PART I																
1		12.90	3.80	61.29	52.49	8.43*	60.92	3.98	1,281	5,541	6,822	−1,682	3,837	2,662	6,499	−939
2				61.12	55.05		63.48	1.42	1,360	5,541	6,901	−1,761	3,601	2,662	6,263	−703
3		12.67	4.01	60.44	50.72		59.15	5.75	1,302	5,541	6,843	−1,703	3,625	2,662	6,287	−727
4	Low Ca intake, periods 4 & 5	12.67	4.02	60.26	42.28		50.71	14.19	895	2,961	3,856	−2,621	2,725	1,992	4,717	843
5				60.38	41.81		50.24	14.66	842	2,961	3,802	−2,568	3,341	1,992	5,333	227
6		12.37	4.23	60.36	49.37		57.80	7.10	936	4,866	5,802	− 662	3,073	2,404	5,477	83
7		11.79	5.15	60.06	51.23		59.66	5.24	1,035	5,541	6,576	−1,436	3,483	2,662	6,145	−585
8		12.25	4.10	59.64	46.04		54.47	10.43	1,020	5,541	6,561	−1,421	2,968	2,662	5,630	− 70
9		12.31	3.95	60.33	48.43		56.86	8.04	965	5,541	6,506	−1,366	2,945	2,662	5,607	− 47
10		12.35	4.15	60.70	47.23		55.66	9.24	923	5,541	6,464	−1,324	2,648	2,662	5,310	250
11	High P intake, periods 11 & 12	10.64	4.80	61.27	40.68		49.11	15.79	430	5,746	6,176	−1,036	5,956	7,419	13,375	1,617
12†		10.70	4.69	63.18	41.65		50.08	14.82	320	5,746	6,066	− 926	7,373	7,419	14,792	200
13		11.19	3.59	63.62	40.52		48.95	15.95	819	5,383	6,202	−1,062	2,848	2,992	5,840	−280
14		10.86	3.20	63.18	43.55		51.98	12.92	939	5,941	6,880	−1,740	2,707	3,181	5,888	−328
15		10.90	3.75	62.97	43.65		52.08	12.82	901	5,941	6,842	−1,702	2,767	3,181	5,948	−388
16	Propylthiouracil, 600 mg. per day, periods 16–21	10.73	3.76	63.00	45.10		53.53	11.37	835	5,941	6,776	−1,636	2,388	3,181	5,569	− 9
17		10.80	3.64	63.19	38.65		47.08	17.82	630	5,941	6,571	−1,431	1,830	3,181	5,011	549
18		11.16	3.59	63.88	42.73		51.16	13.74	611	5,941	6,552	−1,412	2,129	3,181	5,310	250
19		10.86	3.65	64.42	39.14		47.57	17.33	350	5,941	6,291	−1,151	1,929	3,181	5,110	450
20		9.94	3.49	64.91	41.72		50.15	14.75	369	5,646	6,015	− 875	1,852	3,057	4,909	651
21		10.25	3.12	65.41	38.50		46.93	17.97	290	5,646	5,936	− 796	2,085	3,057	5,142	418
22				66.69	43.23		51.66	13.24	207	5,646	5,853	− 713	2,215	3,057	5,272	288
23		10.19	3.41	66.80	41.95		50.38	14.52	238	5,646	5,884	− 744	2,250	3,057	5,307	253
24		10.15	3.30	67.27	42.43		50.86	14.04	288	5,646	5,934	− 794	2,265	3,057	5,322	238
25		10.78	3.93	67.51	52.58		61.01	3.89	310	5,646	5,956	− 816	2,942	3,057	5,999	−439
PART II																
1		10.18	3.49	69.96	51.35	8.85	60.20	3.70	404	5,777	6,181	− 891	3,158	2,729	5,887	−197
2		10.05	3.70	68.98	56.01		64.86	− .96	359	5,777	6,136	− 846	3,737	2,729	6,466	−776
3				68.64	55.03		63.88	.02	337	5,777	6,114	− 824	3,489	2,729	6,218	−528
4		10.05	3.57	68.30	54.65		63.50	.40	384	5,777	6,161	− 871	3,295	2,729	6,024	−334
5	High Ca intake, periods 5–7	9.73	3.58	68.19	56.96		65.81	−1.91	446	11,111	11,557	1,868	2,668	2,966	5,634	56
6		10.27	3.78	68.29	55.60		64.45	− .55	437	13,102	13,539	− 114	2,398	3,474	5,872	−182
7		10.08	3.67	68.29	49.81		58.66	5.24	382	13,611	13,993	− 568	2,234	3,548	5,782	− 92
8		10.67	3.59	68.39	51.48		60.33	3.57	353	6,566	6,919	−1,629	2,825	2,587	5,412	−278
9		10.28	3.65	68.73	48.10		56.95	6.95	326	5,777	6,103	− 813	2,917	2,729	5,646	44
10		10.30	3.80	68.95	52.70		61.55	2.35	337	5,777	6,114	− 824	3,168		5,897	−207

* Average of the total fecal nitrogen excretion for Parts I and II.
† Therapeutic dose of 2.4 mc. of radioactive iodine administered on the fourth day of period 12.

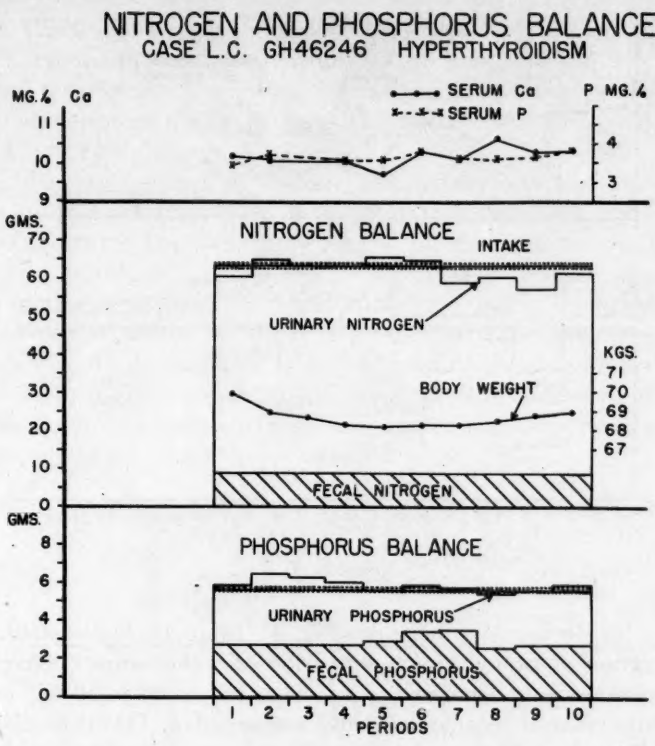

FIGURE 2

males aged 65 or older were able to maintain equilibrium on a diet containing 800 mg. of calcium daily and that calcium balance was positive in all of them when they were receiving 1200 to 1500 mg. daily in the diet.

It is apparent from examination of Table 1 and Figures 3 and 4 that calcium balance was negative throughout the study. The total loss of calcium approximated 39 Gm., or about 3 or 4 per cent of the average calcium content of the total skeleton (17). It would seem reasonable to assume that the patient lost at least this amount while thyrotoxic prior to the investigation, yet his skeleton showed no x-ray evidence of demineralization.

The dissociation between calcium loss and nitrogen and caloric retention during the first ten to eleven weeks of the study was striking. While the cumulative caloric and nitrogen balances were becoming increasingly positive, calcium balance in general was becoming increasingly negative. The negative balance of calcium prior to period 19 (with the exception of the periods of high phosphorus intake) was due to excessive excretion of both urinary and fecal calcium, the elevated urinary calcium contributing sub-

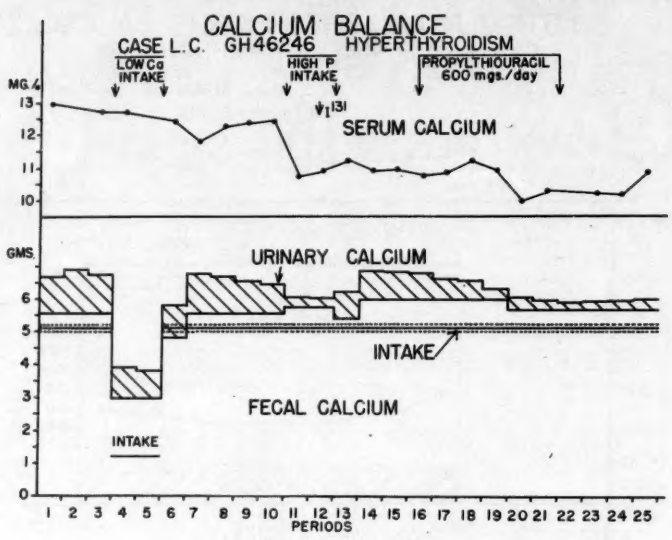

FIGURE 3

stantially to the negative balance (Fig. 3; Table 1). Subsequent to the administration of propylthiouracil (period 16), the urinary excretion of calcium progressively decreased to a level as low as 40 to 50 mg. per day, and calcium balance became decidedly less negative. This is in sharp contrast to the daily excretion of 200 to 300 mg. of calcium during the early periods of the study (Fig. 3) while the patient was thyrotoxic. This hypercalciuria was only slightly affected by rigid restriction of the intake of calcium (periods 4 and 5), which considerably increased the magnitude of the negative balance (Fig. 3).

During the period of high phosphorus intake, however, there was a striking reduction in the renal excretion of calcium, and the balance approached the levels attained during the euthyroid state (Fig. 3 and Table 1). A subsequent return to a normal intake of phosphorus was followed by a prompt increase in the excretion of calcium and in the negative calcium balance. Although urinary loss of calcium was greatest when the patient was hypercalcemic, a marked dissociation between the excretion of calcium and its level in serum was evident after discontinuing the high intake of phosphorus; a rapid threefold increase in urinary calcium occurred (65 to 180 mg.) without a significant change in the level of serum calcium (Fig. 3; Table 1). It seems clear that throughout the study the renal excretion of calcium reflected primarily alterations of the underlying osseous metabolism rather than variations in dietary calcium or its level in the

serum. The fecal excretion of calcium, on the other hand, consistently exceeded the dietary intake of calcium (Figs. 3 and 4) and was relatively constant regardless of the level of thyroidal activity. This gastro-intestinal lag in the absorption of calcium after return to the euthyroid state has been described by others (3).

The metabolic defect in osseous metabolism was less marked when studied during the recurrence of thyrotoxicosis (Part II). Hypercalcemia was not present, urinary calcium excretion increased only slightly over that observed when the patient was euthyroid, and fecal losses were unchanged. In spite of an increase in the intake of calcium to 2.6 Gm. per day, a consistent positive balance could not be attained and the urinary excretion of calcium was not increased (Fig. 4). The positive balance observed during period 5 obviously represented the disequilibrium caused by the sudden increase in calcium intake. This observation emphasizes the danger of misinterpreting short-term changes in calcium balance.

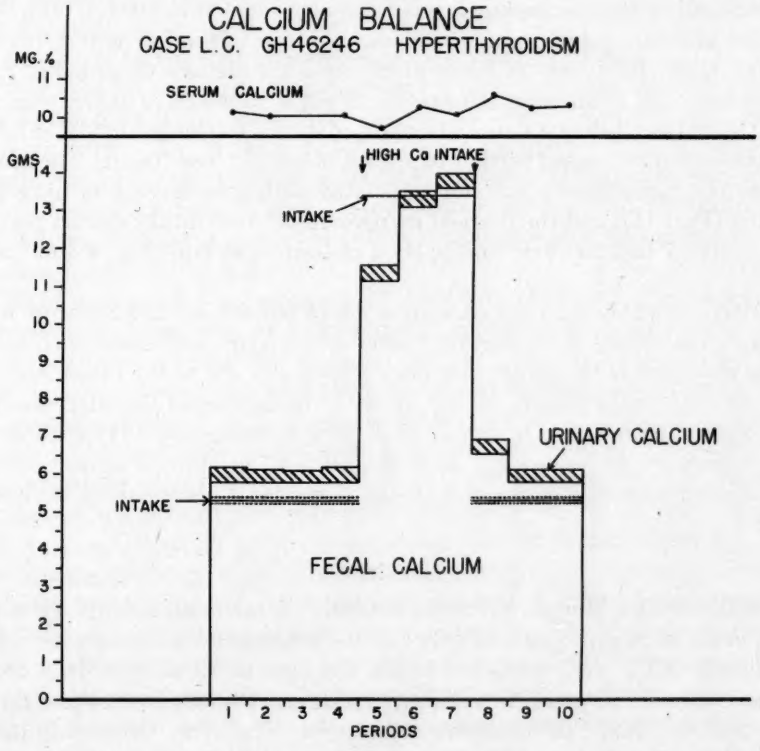

FIGURE 4

The variations in the balance of phosphorus throughout the entire study (Figs. 1 and 2) are readily explained by the simultaneous alterations in calcium and nitrogen balance, assuming a phosphorus to nitrogen ratio of 1.0:14.7 and a phosphorus to calcium ratio of 1.0:2.2 (19).

Serum calcium and phosphorus levels (Figs. 1–4; Table 1)

Hypercalcemia (consistently between 12 and 13 mg. of calcium per 100 ml.) was present prior to the high intake of phosphorus in period 11. The values were unaffected during ten days of rigid calcium restriction (periods 4 and 5). Similarly, while the patient was thyrotoxic but normocalcemic (Part II), the administration of a diet high in calcium did not cause elevation of the serum calcium level. It is apparent that variations in dietary calcium and the nutritional state of the patient, as reflected in the caloric and nitrogen balances, showed no consistent relationship to the level of serum calcium.

In contrast, the high phosphorus intake (periods 11 and 12) caused an abrupt fall to normocalcemic levels (10.6 mg. of calcium per 100 ml.). The failure of serum calcium concentration to return to the previous hypercalcemic levels following withdrawal of the high dietary phosphorus may have been due to an early therapeutic effect of radioactive iodine (Fig. 3). As the patient became definitely euthyroid, the concentration of calcium in the serum decreased further to a level of 9.9 mg. per 100 ml. The recurrence of thyrotoxicosis was not associated with a recurrence of hypercalcemia (Part II), and the marked increase in calcium intake during periods 5 through 7 had no effect on the level of serum calcium (Fig. 4 and Table 1).

Although changes in the concentration of phosphorus in the serum were minor, the values were slightly higher when hypercalcemia was present. This elevation of the serum phosphorus level was due to the hypercalcemia rather than to the thyrotoxicosis, since the recurrence of the latter was not associated with a rise in the level of serum phosphorus. Hypercalcemia secondary to the parenteral administration of calcium is almost always associated with an increase in the level of inorganic phosphate in the serum (19–21).

DISCUSSION

Williams and Morgan (5) noted extensive demineralization of the skeleton in the roentgenograms of only 7 of 195 consecutive patients with thyrotoxicosis. All 7 were women between the ages of 39 and 65. In a radiologic study of 110 cases, Beaumont, Dodds and Robertson (23) found that 11 per cent had "definite" decalcification. The term thyrotoxic osteoporosis has been used to refer to the rare instances of extreme demineraliza-

tion, particularly in cases in which there are such findings as spontaneous compression fractures of the spine (4–6). The extensive histologic studies of Follis (23) and of Askanazy and Rutihauser (24) have disclosed that the bone lesion is not one of osteoporosis but more closely resembles an osteitis fibrosa generalisata. These histologic observations have been confirmed by balance and radioisotope studies (25).

In contrast to the rarity of clinical manifestations, the characteristic abnormality of calcium metabolism—excessive loss in both urine and feces—is frequently observed (3, 7, 8, 25, 26). Aub and associates (3) studied 14 patients with thyrotoxicosis who were given a low calcium diet. The average three-day calcium excretion of the thyrotoxic subjects was 42 mg. per Kg.; that of the normal subjects was 13 mg. per Kg. In the present study, while the patient was receiving a low calcium diet (280 mg. daily), his three-day combined urinary and fecal excretion of calcium averaged 40 mg. per Kg. (Table 1, periods 4 and 5). Beaumont, Dodds and Robertson found excessive losses of calcium in 18 of 33 hyperthyroid patients (22).

In view of the foregoing metabolic observations, it is striking that more subjects do not manifest clinical abnormalities in the skeletal system. The present patient lost at least 3 per cent of his skeletal calcium while under investigation. At that rate, it would be only a matter of time before he would exhibit overt osseous pathologic changes. It must be concluded that "thyrotoxic bone disease" is not seen more frequently because the underlying disorder is corrected before the demineralization proceeds to a clinically demonstrable level.

What pathogenic mechanisms associated with the thyrotoxic state cause the excessive renal and fecal excretion of calcium? That the mechanisms are related in a general way to the level of thyroid activity is evident from the converse observation that the excretion of calcium is abnormally low in hypothyroidism (3, 25). More specifically, the observations in the present and previous studies (3, 25–28) led to the elimination of a number of factors which might adversely affect calcium metabolism. The balance studies of the present and earlier investigations (3, 25, 28) disclosed no causative relationship between undernutrition or protein depletion and the osseous disorder. The administration of large doses of vitamin D have no effect on the excessive calcium loss associated with hyperthyroidism (26). Overactivity of the parathyroid glands cannot be implicated, for excessive losses of calcium occur even when thyrotoxicosis is associated with hypoparathyroidism (27). Moreover, hypermetabolic states other than thyrotoxicosis do not produce a similar defect in calcium metabolism (3).

It would seem that only 2 basic mechanisms could be involved: 1) a direct effect of thyroid hormone on intestinal absorption and renal tubular

reabsorption of calcium, and 2) a direct effect on osseous metabolism. The former cannot be reconciled with the observed data. A drain of skeletal calcium due to a primary action on the kidney and gastro-intestinal tract could not cause the hypercalcemia observed in the present case. Similarly, the increase in the level of serum calcium in *hypoparathyroid* subjects given large doses of thyroid hormone (3, 27) cannot be explained by a direct action of the latter on the renal tubules and gastro-intestinal tract. A loss of skeletal calcium due to a disturbance of the gastro-intestinal tract and kidney would be expected to cause osteomalacia with a lowered level of serum phosphorus. These pathologic and chemical changes have not been observed (3, 22–24).

Urinary calcium, which in the present study was a sensitive indicator of the state of osseous metabolism, was not appreciably altered by either a low or a high intake of calcium. The only 2 experimental or therapeutic procedures which significantly altered the urinary excretion of calcium were the production of the euthyroid state by treatment with radioactive iodine and propylthiouracil, and the administration of a high phosphorus diet (Table 1, periods 11 and 12; and Fig. 3). The marked reduction in the renal excretion of calcium following ingestion of phosphate was as great as that observed when the euthyroid state was attained. This strongly suggests that phosphorus, for the periods of its administration, reversed or counteracted the underlying pathogenic mechanism. The simultaneous fall in serum calcium concentration, when combined with the decrease in urinary calcium, implies an effect of phosphorus at an osseous or at least a prerenal level. It is unlikely that administration of phosphate, acting directly on a renal mechanism causing extensive urinary excretion of calcium, would produce a *decrease* in the concentration of calcium in the serum.

These observations can be best reconciled with the second probable mechanism—a *direct* effect of excessive thyroid hormone on skeletal metabolism. The recent studies of Krane and associates (25), utilizing radioactive calcium and conventional balance techniques, support this contention. These investigators demonstrated that while the total skeletal calcium was reduced, the readily exchangeable pool of labile calcium was increased and was associated with a more rapid rate of skeletal turnover. From their data, utilizing the equations of Bauer, Carlsson and Lindquist (29), they calculated that thyrotoxicosis was associated with an increased rate of osteogenesis. However, the presence of a negative external balance of calcium indicated that bone resorption or destruction exceeded the rate of bone formation. Under these circumstances, the increased loss of urinary and fecal calcium *reflected* the excessive resorptive process.

The increased amount of calcium ion released from the skeleton under

these circumstances obviously must first enter the extracellular pool, and this *per se* would cause an increase in the concentration of serum calcium. However, in the great majority of thyrotoxic subjects there is no elevation of the serum calcium level (3, 6, 22, 23, 25) despite osseous losses which can increase the urinary excretion of calcium to values of 1 Gm. daily (3). In experimental thyrotoxicosis, urinary calcium may increase tenfold without any significant rise in the level of serum calcium (30). It is apparent that in almost all instances the homeostatic mechanisms which maintain the normal level of ionized calcium in the body fluids cope adequately with the excessive load of calcium. In the present case, and in the 8 others with hypercalcemia found in the literature (7–10), the regulatory mechanisms were either overwhelmed or defective. The latter condition is suggested by the following observations: 1) the severity of the thyrotoxicosis and the magnitude of the loss of skeletal calcium was not greater in the hypercalcemic group (7–10) than in the normocalcemic group (3, 25, 26); 2) rigid restriction of the calcium intake had no effect on the hypercalcemia (Fig. 3, Table 1, and (7)); and 3) the present subject was normocalcemic at a time when there was a marked increase in calcium intake, and recurrent thyrotoxicosis did not cause hypercalcemia (Fig. 4). It is interest that when thyrotoxicosis recurred in 2 of the cases previously reported, hypercalcemia did not recur (10).

The fundamental homeostatic factors controlling the level of ionized calcium in the body fluids are the steady state of equilibrium between the extracellular calcium ion and the surface of the bone crystals (31) and the renal clearance of filtrable calcium. The former and probably the latter are controlled primarily by the level of circulating parathyroid hormone. Removal of the parathyroid glands causes a net movement of calcium ion into bone (31), a fall in the level of serum calcium, and a simultaneous *increase* in the renal *clearance* of calcium (32–34). Therefore, a rise in extracellular calcium secondary to thyrotoxicosis would cause an inhibition of the release of parathyroid hormone which, in turn, would cause a decrease in serum calcium concentration and an increase in renal calcium excretion in spite of the lower or normal serum level. This mechanism may well have been defective in the present case, since hypercalcemia developed and the magnitude of the hypercalciuria was relatively low (average, 205 mg. per day; Table 1) in view of the elevated serum level. That homeostatic alteration in parathyroid activity is important is evident from the observation that either administration of thyroid or thyrotoxicosis can induce elevation of the level of serum calcium in parathyroidectomized human subjects or animals, but not when the parathyroid glands are intact (3, 27, 30). The slight hyperphosphatemia observed in the present case during the hypercalcemic phase (Table 1) was not necessarily due to inhibition of para-

thyroid activity, for an increase in serum phosphorus concentration accompanies hypercalcemia regardless of the presence or absence of the parathyroids (19, 20, 21) and the absence of renal impairment.

REFERENCES

1. Werner, S. C., Ed.: The Thyroid. New York, Paul B. Hoeber, Inc., 1955.
2. Talbot, M. B.; Sobel, E. H.; McArthur, J. W., and Crawford, J. D.: Functional Endocrinology from Birth through Adolescence. Cambridge, Mass., Harvard University Press, 1952.
3. Aub, J. C.; Bauer, W.; Heath, C., and Ropes, M.: Studies of calcium and phosphorus metabolism. III. The effects of the thyroid hormone and thyroid disease, *J. Clin. Invest.* **7**: 97, 1929.
4. Bartels, E. C., and Haggart, G. E.: Osteoporosis in hyperthyroidism: report of two cases with compression fractures of the vertebrae, *New England J. Med.* **219**: 373, 1938.
5. Williams, R. H., and Morgan, H. Q.: Thyrotoxic osteoporosis, *New Interntl. Clin.* **2**: 48, 1940.
6. Laake, H.: Osteoporosis in association with thyreotoxicosis, *Acta med. scandinav.* **151**: 229, 1955.
7. Stanley, M. M., and Fazekas, J.: Thyrotoxicosis simulating hyperparathyroidism, *Am. J. Med.* **7**: 262, 1949.
8. Puppel, I. D.; Gross, H. T ; McCormick, E. K., and Herdle, E.: The rationale of calcium, phosphorus, and vitamin D therapy in clinical hyperthyroidism, *Surg., Gynec. & Obst.* **81**: 243, 1945.
9. Miller, E. S., and Evans, L. R.: Simultaneous hyperfunction of the thyroid and parathyroid glands, *New England J. Med.* **227**: 949, 1942.
10. Rose, E., and Boles, R. S., Jr.: Hypercalcemia in thyrotoxicosis, *Med. Clin. North America* **37**: 1715, 1953.
11. Kochakian, C., and Fox, R.: Microdetermination of calcium by titration of the oxalate with ammonium hexanitratocerate, *Indust. & Eng. Chem.* **16**: 762, 1944.
12. Folin, O., and Wright, L. E.: A simplified macro-Kjeldahl method for urine, *J. Biol. Chem.* **38**: 461, 1919.
13. Fiske, C., and SubbaRow, Y.: The colorimetric determination of phosphorus, *J. Biol. Chem.* **66**: 375, 1925.
14. Washburn, M., and Shear, M.: Composition of bone. XIII. Direct gravimetric determination of calcium, magnesium and phosphorus, *J. Biol. Chem.* **99**: 21, 1932.
15. Mitchell, H. H., and Hamilton, T. S.: The dermal excretion under controlled environmental conditions of nitrogen and minerals in human subjects with particular reference to calcium and iron, *J. Biol. Chem.* **178**: 345, 1949.
16. Bogdonoff, M. D.; Shock, N. W., and Nichols, M. P.: Calcium, phosphorus, nitrogen, and potassium balance studies in the aged male, *J. Gerontol.* **8**: 272, 1953.
17. Howard, J. E.: *in* Metabolic Interrelations, Trans. of the 5th Conference, New York, Josiah Macy, Jr. Foundation, 1953, p. 23.
18. Reifenstein, E. C., Jr.; Albright, F., and Wells, S. L.: The accumulation, interpretation, and presentation of data pertaining to metabolic balances, notably those of calcium, phosphorus, and nitrogen, *J. Clin. Endocrinol.* **5**: 367, 1945.
19. Howard, J. E.; Hopkins, T. R., and Connor, T. B.: On certain physiologic responses to intravenous injection of calcium salts into normal, hyperparathyroid and hypoparathyroid persons, *J. Clin. Endocrinol. & Metab.* **13**: 1, 1953.

20. NORDIN, B. E. C., and FRASER, R.: The effect of intravenous calcium on phosphate excretion, *Clin. Sc.* **13**: 477, 1954.
21. CHEN, P. S., JR., and NEUMAN, W. F.: Renal excretion of calcium by the dog, *Am. J. Physiol.* **180**: 623, 1955.
22. BEAUMONT, G. E.; DODDS, E. C., and ROBERTSON, J. D.: Calcium and phosphorus metabolism in thyrotoxicosis, *J. Endocrinol.* (British) **2**: 237, 1940.
23. FOLLIS, R. H., JR.: Skeletal changes associated with hyperthyroidism, *Bull. Johns Hopkins Hosp.* **92**: 305, 1953.
24. ASKANAZY, M., and RUTISHAUSER, E.: Die Knochen der Basedow-Kranken, *Virchows Arch. f. path. Anat.* **291**: 653, 1933.
25. KRANE, S. M.; BROWNELL, G. L.; STANBURY, J. B., and CORRIGAN, H.: The effect of thyroid disease on calcium metabolism in man, *J. Clin. Invest.* **35**: 874, 1956.
26. TIBBETTS, D. M.; MCLEAN, R., and AUB, J. C.: Studies of calcium and phosphorus metabolism. XX. The high calcium excretion in exophthalmic goiter is not due to vitamine D deficiency, *J. Clin. Invest.* **11**: 1273, 1932.
27. COPE, O., and DONALDSON, G. A.: Relationship of thyroid and parathyroid glands to calcium and phosphorus metabolism. Study of a case with coexistent hypoparathyroidism and hyperthyroidism, *J. Clin. Invest.* **16**: 329, 1937.
28. GREEN, J., and LYALL, A.: Calcium metabolism in hyperthyroidism. The effect of thiouracil, *Lancet* **1**: 828, 1951.
29. BAUER, G. C. H.; CARLSSON, A., and LINQUIST, B.: Evaluation of accretion, resorption and exchange reactions in the skeleton, *in* Kungl. Fysiografiska Sällskapet, Lund Förhandlingar, 1955, vol. 25, p. 1.
30. LOGAN, M. A.; CHRISTENSEN, W. R., and KIRKLIN, J. W.: Thyroid and parathyroid hormone effects on calcium and phosphorus metabolism, *Am. J. Physiol.* **135**: 419, 1942.
31. HOWARD, J. E.: Present knowledge of parathyroid function, with especial emphasis upon its limitations, *in* Ciba Foundation Symposium on Bone Structure and Metabolism, ed. by G. Wolstenholme and C. O'Connor. Boston, Little, Brown & Co., 1956, p. 206.
32. TALMAGE, R. V., and KRAINTZ, F. W.: Progressive changes in renal phosphate and calcium excretion in rats following parathyroidectomy or parathyroid administration, *Proc. Soc. Exper. Biol. & Med.* **87**: 263, 1954.
33. TALMAGE, R. V.; KRAINTZ, F. W., and BUCHANAN, G. D.: Effect of parathyroid extract and phosphate salts on renal calcium and phosphate excretion after parathyroidectomy, *Proc. Soc. Exper. Biol. & Med.* **88**: 600, 1955.
34. KLEEMAN, C. R.: Unpublished observations.

SEVERE UTERINE BLEEDING AND DEGENERATIVE SKELETAL-MUSCLE CHANGES IN UNRECOGNIZED MYXEDEMA

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ABSTRACT

Two cases of severe uterine bleeding associated with unrecognized myxedema are reported. Degenerative changes in skeletal muscles were suggested by the results of electromyographic studies and demonstrated by biopsy of a muscle prior to treatment in one patient. The uterine bleeding stopped promptly and a normal menstrual cycle was re-established in both patients after treatment with desiccated thyroid.

DISTURBANCES in thyroidal function in premenopausal women are often associated with abnormalities of menstruation. Either menorrhagia or amenorrhea may develop in patients with spontaneous or postoperative myxedema. However, when amenorrhea occurs in a premenopausal woman with myxedema, the diagnosis of hypopituitarism with secondary hypothyroidism should be considered and excluded. In his book, Means (1) stated: "Menorrhagia may be sufficiently impressive in ordinary myxedema so that in several cases that have come to our attention patients have actually had a dilation and curettage for it when all they needed was desiccated thyroid for treatment."

We are reporting 2 cases of profuse uterine bleeding with severe anemia secondary to unrecognized primary myxedema, in which prompt cessation of uterine bleeding and restoration of normal menstrual cycles followed the oral administration of desiccated thyroid. Significant degenerative changes in the skeletal muscles were suggested by the results of electromyography and were demonstrated by muscle biopsy prior to treatment in one of these patients.

REPORT OF CASES

Case 1. A 55-year-old white woman was hospitalized in January 1956, for treatment of profuse uterine bleeding of three weeks' duration. Her menstrual periods had begun when she was 13 years of age and had recurred every twenty-six to twenty-eight days,

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with a moderate flow lasting three to four days. During the six-month period preceding her examination, the duration of flow had increased, lasting from six to ten days. Intermenstrual spotting had not occurred. One week prior to examination at the Mayo Clinic, she was hospitalized elsewhere and received 5 blood transfusions.

In 1953, the patient had noted the onset of progressive fatigue, dryness of the skin, puffiness of the face and hoarseness. There was no history suggestive of acute or subacute thyroiditis.

Physical examination revealed a blood pressure of 112 mm. Hg systolic and 70 mm. diastolic. Her weight was 265 pounds (120 Kg.). The pulse rate was 60 beats per minute. The skin was extremely dry, and her face was puffy and had a waxy pallor. Thinning of the outer third of the eyebrows was noted. The recovery phase of the tendon-stretch reflexes was delayed. Pelvic examination showed profuse uterine bleeding. The cervix appeared normal; the uterus was not enlarged and no adnexal masses were palpable. The initial clinical impression was primary myxedema with secondary menorrhagia.

Laboratory studies yielded the following results: The hemoglobin level was 8.6 Gm. per 100 ml. of blood, and the erythrocyte count was 3,000,000 cells per cu. mm. The bleeding time was 3 minutes, coagulation time 8 minutes and prothrombin time 21 seconds (normal, 17 to 19 seconds). The platelet count was 157,000 cells per cu. mm. of blood. Study of stained blood smears showed moderate hypochromasia, polychromasia and poikilocytosis. The blood urea level was 52 mg. per 100 ml. and cholesterol, 240 mg. per 100 ml. The basal metabolic rate was −22 per cent.

In the absence of hypertension, cardiac enlargement or angina pectoris, and in view of the severe bleeding, the patient was given 3 grains of uncoated desiccated thyroid (U.S.P.) daily. After five days of treatment, the uterine bleeding ceased. At the end of one week, the dosage of thyroid was reduced to 2 grains daily. The basal metabolic rate was −12 per cent on the twelfth day of treatment. Immediate improvement of the anemia did not occur; therefore, she was given 1,000 ml. of blood, after which the hemoglobin concentration increased to 10.6 Gm. per 100 ml.

At the time of dismissal, the patient was greatly improved. Her skin was less dry, and the facial edema less apparent. She was instructed to take a 1,000-calorie reducing diet, 2 grains of desiccated thyroid daily, and 5 grains of ferrous sulfate three times a day.

She returned in May, 1956. The puffiness of her face had disappeared and the texture of her skin was normal. Her menstrual periods had become normal again, occurring every twenty-six to twenty-eight days and lasting four to five days, with moderate flow. Her weight was 199 pounds (90 Kg.). The basal metabolic rate was 0, and the hemoglobin level was 11.5 Gm. per 100 ml.

Case 2. A 35-year-old white woman was hospitalized on April 30, 1956, because of severe exsanguination secondary to profuse uterine bleeding. She had been referred for consideration of a pelvic operation. Her menstrual periods had begun at age 11 and had recurred every twenty-six to twenty-eight days, with a moderately excessive flow, at times lasting up to nine days. Pregnancies in 1938, 1942, 1949 and 1950 had terminated with spontaneous deliveries of normal infants at term, with uncomplicated postpartum courses. Lactation was normal and the menstrual periods had begun on every occasion from two to six months post partum. Dilation and curettage of the uterus had been carried out on three occasions between 1946 and 1949 because of recurrent intermenstrual bleeding.

After the birth of her fourth child in 1950, her menstrual periods had been regular until intermenstrual bleeding began to recur in January 1954. Another dilation and curettage was performed in January 1955, following which the menstrual periods were

regular for one year. Excessive uterine bleeding was present for four of the seven weeks between February 12, 1956, and March 30, 1956. From March 30 until admission to the hospital, the vaginal flow was intermittent but profuse, requiring five or six changes of the perineal pad per day.

In 1950, after the birth of her fourth child, she had noted the onset of severe fatigue after minimal exertion. In 1953, puffiness of the face had become apparent, and the lethargy and fatigue had become more disabling.

Physical examination revealed severe weakness and pallor. The blood pressure was 100 mm. Hg systolic and 70 mm. diastolic. Puffiness of the eyelids and face was pronounced. The skin was dry, and axillary hair was sparse. The recovery phase of the tendon-stretch reflexes was greatly delayed. No thyroid tissue was palpable in the neck. Results of pelvic examination were normal except for profuse uterine bleeding and minimal erosion of the cervix. The clinical diagnosis was primary myxedema with secondary menometrorrhagia.

Blood studies showed an erythrocyte count of 1,520,000 per cu. mm. and a hemoglobin level of 4.3 Gm. per 100 ml. The patient was immediately given a transfusion of 500 ml. of whole blood. On the following day the erythrocyte count was 1,800,000, a leukocyte count was 7,800 and the hemoglobin level was 5.6 Gm. per 100 ml. Smears of the peripheral blood showed abundant polychromasia, anisocytosis and microcytosis, with adequate platelets; rare spherocytes and myelocytes were present. Roentgenograms of the head and thorax were normal. The basal metabolic rate was −29 per cent, and the value for serum protein-bound iodine was 1.0 μg. per 100 ml. Studies utilizing radioactive iodine revealed only 2 per cent of the tracer dose present in the thyroid gland in six hours and 3 per cent in twenty-four hours. Parenteral administration of 10 units of thyroid-stimulating hormone produced no change in the thyroidal uptake of I^{131}. The serum protein concentrations were normal, and the value for serum transaminase was 1.85 micromoles per hour per milliliter of serum (the mean plus 2 standard deviations of normal values for women is 1.36) (2).

Prior to treatment with desiccated thyroid, electromyographic studies were made and biopsy of the left soleus muscle was performed. The patient then was given 1½ grains of desiccated thyroid (U.S.P.) daily, which was increased to 3 grains on the fourth day of treatment. The uterine bleeding stopped on the seventh day after replacement therapy with desiccated thyroid was begun. After two weeks of treatment, the basal metabolic rate was −6 per cent and the serum protein-bound iodine level was 3.5 μg. per 100 ml. She was advised to continue 3 grains of desiccated thyroid daily. On the day of dismissal, the hemoglobin level was 10.7 Gm. per 100 ml. and the erythrocyte count was 3,980,000 per cu. mm.

She returned in August 1956. Her menstrual periods were now regular, recurring every twenty-seven to twenty-eight days, with a moderate flow lasting for four days. The texture of the skin was normal, as was the recovery phase of the tendon-stretch reflexes. There was a scanty new growth of axillary hair. Results of pelvic examination were normal. The basal metabolic rate was +15 per cent, the serum protein-bound iodine level 4.6 μg. per 100 ml., hemoglobin level 10.5 Gm. per 100 ml., and erythrocyte count 4,400,000 per cu. mm. The patient was advised to decrease the dose of thyroid to 2 grains per day.

COMMENT

Both these patients were referred for consideration of surgical treatment for intractable profuse uterine bleeding. Severe menorrhagia lasting from

six to ten days had been present during the six months preceding the admission of the first patient to the hospital. After the clinical recognition of myxedema, it was realized that her bleeding might be due to the hypothyroidism. For this reason, operation was deferred until the patient could be restored to a euthyroid state. After five days of oral treatment with desiccated thyroid, the uterine bleeding ceased. The menstrual pattern subsequently returned to normal with regard to periodicity and flow.

Despite the fact that the second patient had noted intermenstrual spotting for many years, the myxedema in all probability began after her last pregnancy in 1950. There was nothing to suggest anterior-pituitary insufficiency, since the patient lactated and cyclic menstrual periods were reestablished following parturition. She was extremely exsanguinated on admission, as shown by the clinical picture of mild shock and severe anemia. Her response to desiccated thyroid was most gratifying, since the uterine bleeding stopped after seven days of therapy. A checkup after three months of treatment revealed that she was clinically euthyroid. A remarkable change had taken place in the facies (Fig. 1). Normal menses had returned.

The reported incidence of menorrhagia in myxedema varies from 32 to

a b

FIG. 1. Appearance in Case 2. **a.** Before treatment. **b.** After three months of treatment with desiccated thyroid.

80 per cent. Means (1) noted menorrhagia in 32 per cent of 41 premenopausal patients with myxedema. Gardiner-Hill and Smith (3) reported menorrhagia in 80 per cent of their series of 23 cases of myxedema, and Lerman (4) mentioned an incidence of 80 per cent in premenopausal patients with myxedema. Lisser and Anderson (5), Means (1) and Chanton and Hargrove (6) have reported cases similar to ours, in which the patient with untreated myxedema sought medical care primarily for menorrhagia or metrorrhagia.

Goldsmith and associates (7) and Lederer (8) have reported studies of endometrial biopsies, vaginal smears, curves of the rectal temperature and urinary excretion of pregnanediol in premenopausal patients with myxedema. These workers concluded that untreated primary myxedema may be associated with continuous estrogenic stimulation of the endometrium, with failure of ovulation and of corpus-luteum formation. Esselborn and Goldsmith (9) recently presented unpublished data concerning the efficacy of injections of chorionic gonadotropin in stimulating ovulation and corpus-luteum formation in myxedematous females. Similar findings in thyroidectomized rabbits led Chu (10) to attribute the failure of ovulation and corpus-luteum formation to inadequate stimulation by the pituitary luteinizing hormone. Statland and Lerman (11) studied urinary excretion of gonadotropin before and after treatment of premenopausal women who had myxedema. The observed variations were not thought to be significant. Failure of ovulation does not invariably accompany untreated primary myxedema, however, since Hodges and co-workers (12) found a total of 17 successful term pregnancies in such patients reported up to 1952.

Pretreatment electromyographic examination of the calf, anterior tibial, biceps brachii, first dorsal interosseous and sacrospinal muscles in the second patient did not reveal spontaneous fibrillation of the type seen in denervated muscle nor did it demonstrate fasciculation. The motor-unit action potentials observed during voluntary contraction of these muscles were essentially normal. However, the examination did reveal in some areas abnormal irritability of muscle fibers to mechanical stimulation with the needle electrode. Movement of the electrode, particularly in the calf and sacrospinal muscles, occasionally evoked trains of positive waves (Fig. 2). This was less evident in an electromyographic examination made three months after treatment with desiccated thyroid was begun.

The abnormal irritability of the muscle, although minimal in degree, resembled in some respects that associated with degeneration of muscle fibers in thyroidectomized rabbits (13). As a consequence, biopsy of the left soleus muscle was performed and reported by Dr. G. P. Sayre, of the Section of Pathologic Anatomy. Diffusely scattered throughout the muscle

fascicles were muscle fibers in varying stages of degeneration, and occasional regenerating fibers. The degeneration was first apparent as diffuse swelling of part of a fiber, with gradual loss of the structure of the fibrils. These became homogeneous and then appeared to fuse into a homogeneous mass. Nucleated cells, apparently macrophages, although possibly sarcolemmal nuclei, invaded the necrotic fibers, with evidence of peripheral vacuolation around the cells. The structure of the fibers then became granular and the number of phagocytes increased until only the outline of

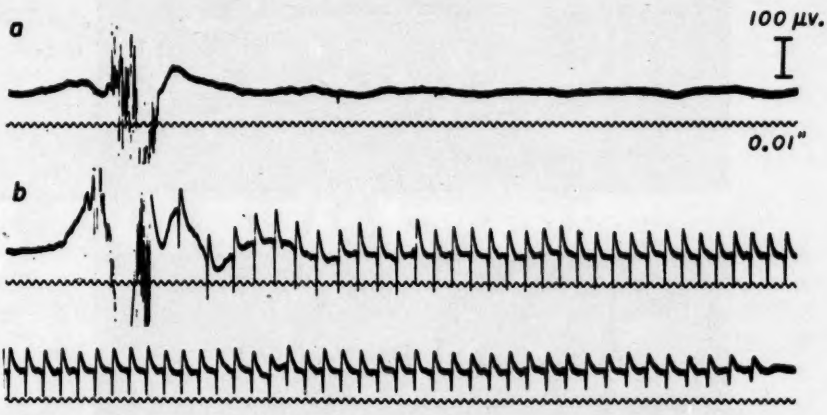

FIG. 2. Electrical activity evoked by movement of a needle electrode in the sacrospinalis muscle. **a.** Normal appearance. A brief outburst of activity lasts but little longer than the movement of the needle. **b.** Appearance in Case 2. A train of "positive sharp waves" follows movement of the needle. A downward deflection indicates a change of potential in the positive direction of the needle.

the fibers remained, distended with phagocytes (Fig. 3). Associated with the degenerating fibers were occasional regenerating fibers with basophilic cytoplasm and large vesicular nuclei and prominent nucleoli. These fibers appeared to occupy part of the space of previously degenerated fibers. No obvious growth cap was present. The capillaries in the zones of degeneration and regeneration were numerous, and endothelial cells were prominent. With the exception of the phagocytes within the degenerating muscle fibers, evidence of a generalized cellular reaction was absent. The picture was that of primary degeneration of muscle fibers, nonspecific in type.

Conduction studies of the left ulnar nerve revealed a normal electrical response of the hypothenar and adductor pollicis muscles to stimulation of the nerve. The conduction velocity of the motor fibers of the nerve was normal (54 meters per second). No significant change occurred after treat-

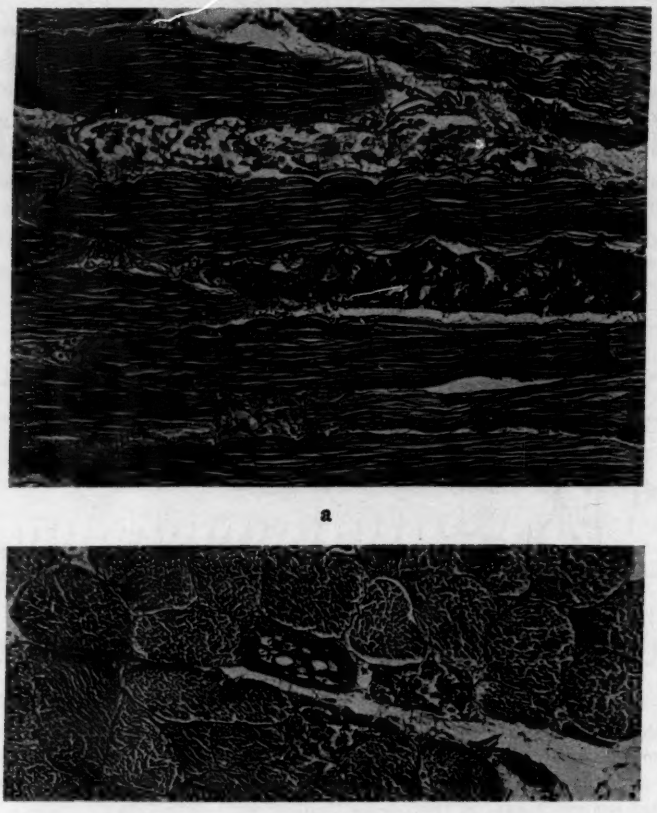

FIG. 3. **a.** Longitudinal section of soleus muscle in Case 2, with a fiber almost completely replaced by phagocytes (upper), and a fiber undergoing hyaline degeneration with vacuolization and infiltration by a few phagocytes. **b.** Cross-section of same muscle with 3 fibers in varying stages of vacuolar degeneration and phagocytosis. (Both, hematoxylin and eosin; ×175.)

ment. On the other hand, the mechanical twitch of the adductor pollicis muscle in response to a single stimulus applied to the ulnar nerve was prolonged prior to treatment and became shorter in duration with administration of desiccated thyroid. Similarly, in measurements of the speed of contraction and relaxation of the calf muscle in the Achilles-tendon reflex, the mechanical response prior to therapy was greatly prolonged, particularly in the relaxation phase, but returned to the normal range as treatment became effective (Fig. 4).

These studies illustrate effects of myxedema on both the function and

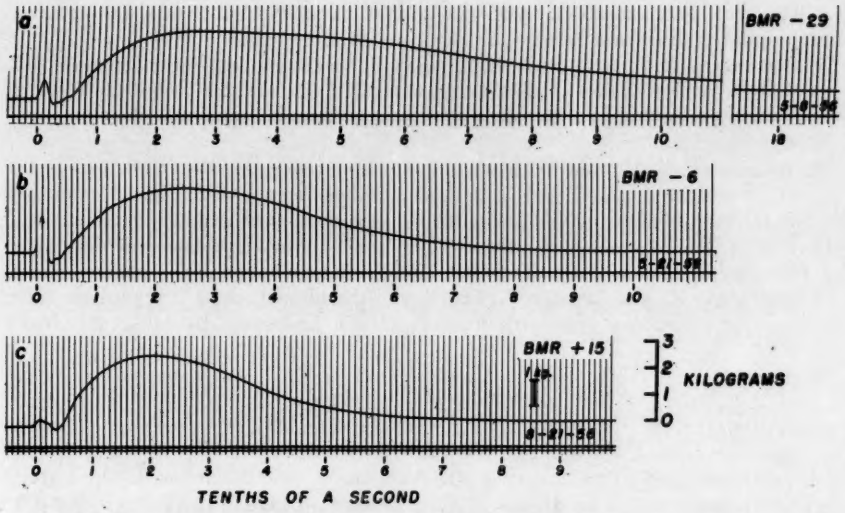

FIG. 4. Records of the ankle jerk (Achilles-tendon reflex) in Case 2. **a.** Before treatment. **b** and **c.** During treatment of myxedema. The initial deflection in the record of muscular contraction is produced by the tap on the Achilles tendon, which initiates the reflex. Vertical lines mark 0.01-second intervals.

structure of skeletal-muscle fibers. Lambert and associates (14) have shown that slowing of the contraction and relaxation of muscles is observed commonly in myxedema and probably occurs to some degree in all patients with myxedema. Changes in the structure of muscle fibers are seen less consistently, and many of those described have been considered of doubtful significance (15). However, degenerative changes in advanced or long-standing myxedema have been observed by Foster and Barr (16) and others, not only in skeletal-muscle fibers but also in cardiac muscle and smooth muscle.

REFERENCES

1. MEANS, J. H.: The Thyroid and Its Diseases, ed. 2. Philadelphia, J. B. Lippincott Co., 1948, 571 pp.
2. FLEISHER, G. A.; WAKIM, K. G., and GOLDSTEIN, N. P.: Glutamic-oxalacetic transaminase and lactic dehydrogenase in serum and cerebrospinal fluid of patients with neurologic disorders, *Proc. Staff Meet., Mayo Clin.* **32**: 188–197 (Apr. 17) 1957.
3. GARDINER-HILL, H., and SMITH, J. F.: Influence of disease of thyroid on menstruation, *J. Obst. & Gynaec. Brit. Emp.* **34**: 701–711, 1927.
4. LERMAN, J.: The thyroid in gynecology, *in* Meigs, J. V., and Sturgis, S. H.: Progress in Gynecology. New York, Grune & Stratton, 1950, vol. 2, pp. 257–260.
5. LISSER, H., and ANDERSON, E. M.: Three cases of adult myxedema in women, reported for purpose of calling attention to their widely different symptomatology and clinical findings, *Endocrinology* **15**: 365–381 (Sept.–Oct.) 1931.

6. CHANTON, E. F., and HARGROVE, M.D.: Myxedema: report of 10 cases from general hospital, *New Orleans M. & S. J.* **100:** 546–551 (June) 1948.
7. GOLDSMITH, R. E.; STURGIS, S. H.; LERMAN, J., and STANBURY, J. B.: The menstrual pattern in thyroid disease, *J. Clin. Endocrinol. & Metab.* **12:** 846–855 (July) 1952.
8. LEDERER, J.: Etude de la ménorragie myxedémateuse, *Ann. endocrinol.* **15:** 397–400, 1954.
9. ESSELBORN, V. M., and GOLDSMITH, R.: Studies concerning the mechanism of the effect of thyroid insufficiency on ovulation in the human. (Abstr. No. 122) Program of the 39th Meeting of The Endocrine Society, New York, May 30–June 1, 1957.
10. CHU, J. P.: Influence of the thyroid gland on pituitary gonadotrophic activity in the rabbit, *Endocrinology* **34:** 90–102 (Feb.) 1944.
11. STATLAND, H., and LERMAN, J.: Function of the adrenal cortex in myxedema, with some observations on pituitary function, *J. Clin. Endocrinol.* **10:** 1401–1416 (Nov.) 1950.
12. HODGES, R. E.; HAMILTON, H. E., and KEETTEL, W. C.: Pregnancy in myxedema, *A.M.A. Arch. Int. Med.* **90:** 863–868 (Dec.) 1952.
13. LAMBERT, E. H., and SAYRE, G. P.: Myopathy in rabbits following thyroidectomy, (abstract) *Am. J. Physiol.* **183:** 636–637 (Dec.) 1955.
14. LAMBERT, E. H.; UNDERDAHL, L. O.; BECKETT, S., and MEDEROS, L. O.: A study of the ankle jerk in myxedema, *J. Clin. Endocrinol.* **11:** 1186–1205 (Oct.) 1951.
15. ADAMS, R. D.; DENNY-BROWN, D., and PEARSON, C. M.: Diseases of Muscle. New York, Paul B. Hoeber, Inc., 1953, 556 pp.
16. FOSTER, M., and BARR, D. P.: Myxedema: record of an autopsied case with special emphasis upon lesions of muscles, *J. Clin. Endocrinol.* **4:** 417–426 (Sept.) 1944.

STUDIES ON THE PATHOGENESIS OF THE ANEMIA OF HYPOTHYROIDISM

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ABSTRACT

The blood of 11 adult hypothyroid patients was labeled with radioactive chromium^{51}. The affinity of the erythrocytes for radiochromium *in vitro* was 54.5 per cent on the average, and the apparent half-life of Cr^{51}-labeled erythrocytes *in vivo* was 13.8 days—results distinctly different from the normal values of 80 per cent and 24 days previously established in our laboratory. The frequency of anemia in hypothyroidism may be due to the shortened life span of the erythrocytes.

THE influence of the endocrine glands on the formed elements of the blood has recently received extensive study. Axelrod and Berman (1) reviewed all published data on the relationship between thyroid function and hematopoiesis available by 1951. Much of the literature was found to be fragmentary and contradictory. The information, derived from morphologic studies of the peripheral blood and bone marrow of hypothyroid experimental animals as well as human beings, can be summarized as follows:

1. Anemia is a common finding in hypothyroidism. It is of the simple macrocytic type in uncomplicated cases, but can be hypochromic if achylia gastrica is also present and leads to iron deficiency. True Addisonian pernicious anemia may be superimposed, if the anti-pernicious anemia factor becomes deficient because of lack of intrinsic factor. There are no significant changes in the leukocytes and platelets.

2. Hypoplasia of the bone marrow is present in hypothyroid states, and affects almost equally all the myeloid elements. A normal ratio between red and white cells is thus preserved. The total quantity of active marrow, however, cannot be seriously depleted; otherwise there would be a significant depression of all cellular elements in the peripheral blood.

The anemia of uncomplicated hypothyroidism obviously is not due to bone marrow hypoplasia alone. Neither is it due to blood loss. The life span of the red cell, therefore, could be a factor contributing to the anemia. Our present report deals with a study of red-cell turnover in 11 cases of adult myxedema, using chromium^{51}.

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METHODS

For many years the normal life span of human red cells was believed to be approximately 120 days, as determined by Ashby's biologic method of differential agglutination counting of transfused blood (2). The advent of radioisotopes permitted *in vitro* labeling of red cells (3). A practical method for studying the life span of erythrocytes was found when Cr^{51} proved to be a label which, for all practical purposes, remained within the red cells until their disappearance from the circulation. The disappearance of re-injected labeled cells was determined by simply measuring the decrease in radioactivity of the peripheral blood (4–6). Erythrocyte survival determinations were then performed and compared simultaneously in some cases with those performed by Ashby's method (7). The results corroborated the fact that 120 days was the average for the life of a normal human red cell. More important, perhaps, was the finding that the rate of loss and the total decline of Cr^{51} activity were reproducible in healthy subjects. This reproducibility firmly established radiochromium survival time as an index of the life span of erythrocytes (8, 9).

We studied the correlation between red-cell affinity for Cr^{51} (*in vitro*) and the rate of disappearance from the peripheral circulation *in vivo*. This survival curve is plotted as remaining blood radioactivity against time. Two aspects of the graph thus obtained have been closely analyzed: 1) the time it takes for the initial radioactivity to decrease by 50 per cent, called the apparent Cr^{51} half-life (T), and 2) the rate of change in blood radioactivity during the first day of study. The latter is represented by the tangent to the initial slope of the curve. This is obtained by joining the plotted points for the initial radioactivity (P_0) and the radioactivity present after twenty-four hours (P_1). The projection of this tangent will intersect the time axis at a point (V) which we consider to reflect the viability of the red cells under the conditions of survival for the first day of study. This viability value (V), expressed in terms of days, is readily determined without need of graphic plotting. The mathematical formula is:

$$V = \frac{P_0}{P_0 - P_1} \quad \text{or} \quad \frac{100}{100\% - P_1}$$

The red cells of 9 normal individuals had a mean *in vitro* Cr^{51} uptake (U) of 80 per cent; their average apparent Cr^{51} half-life (T) was 24 days; and the viability value (V) approximated 10 days. These criteria serve as reference standards in our present work.

CLINICAL DATA

Eleven adult patients exhibiting definite clinical and laboratory evidence of hypothyroidism were studied. Eight of them had spontaneous primary myxedema; 2 had postoperative myxedema, and 1 had secondary

hypothyroidism. There were 9 females and 2 males; only 4 were considered of pure white racial origin.

All had normal total blood volume. The red cell masses varied with the degree of anemia present. Six patients had peripheral red blood cell counts below 4 million per cu. mm. and hemoglobin levels below 12 Gm. per 100 ml. All but 2 had hematocrit readings below 40. The lowest values recorded were 2,900,000 red blood cells per cu. mm., 8.7 Gm. of hemoglobin per 100 ml., and a hematocrit reading of 28. The erythrocyte color index averaged 0.85. Except for moderate hypochromia in 2 patients who had intestinal uncinariasis (hookworm), the mean cell volume, hemoglobin content, and hemoglobin concentration values revealed no gross abnormalities. Reticulocyte counts averaged 0.85 per cent. Red cell fragility was normal. No developing antibodies were elicited by the Coomb's technique. The electrophoretic pattern for hemoglobin was not abnormal. Serum bilirubin and total protein levels were normal. Paper electrophoretic partition of the latter, however, revealed a frequent increase in the gamma globulin fraction at the expense of the albumin component, thus reducing the A/G ratio in such cases to around 1.2. Only 1 of the patients with hookworm infestation had a low serum iron level of 42 mg. per 100 ml. Serum copper values were normal. The blood level of total cholesterol averaged 295 mg. per 100 ml., with a maximum of 415 mg. and a minimum of 179 mg. in the case of secondary myxedema. Blood phospholipid, sodium and potassium concentrations were normal. No patient passed blood in the stools or urine. The 24-hour urinary excretion of 17-ketosteroids averaged 6.6 mg. for females and 6.1 mg. for males.

As shown in Table 1, the mean red cell uptake (U) of Cr^{51} *in vitro* was 54.5 per cent on the average (maximum 64 per cent and minimum 46 per cent) and the mean apparent Cr^{51} half-life (T) *in vivo* was 13.8 days (maximum 19 days, and minimum 6 days in the case of secondary myxedema). Viability values (V) below 10 days were found in all cases.

Five euthyroid subjects received the Cr^{51}-labeled blood from 5 of the hypothyroid patients. The apparent half-life of the labeled cells in such normal environment was 12.8 days, and the viability was less than 10 days.

The Cr^{51} uptake *in vitro* was rechecked in 2 patients with hypothyroidism as soon as they became clinically euthyroid during treatment with oral 3,5,3′-triiodothyronine. Values of 78 per cent and 64 per cent were obtained. Simultaneous peripheral red cell counts and hemoglobin levels were also closer to normal values.

DISCUSSION

These findings reveal the fact that the life of the red cell in hypothyroidism is shortened. This cellular characteristic seems to be constant in patients with deficient thyroid function. The duration of survival is uninflu-

TABLE 1. AVERAGE UPTAKE OF Cr^{51} BY RED CELLS *in vitro* AND APPARENT HALF-LIFE OF Cr^{51}-LABELED RED CELLS *in vivo* COMPARED IN EUTHYROID AND HYPOTHYROID SUBJECTS

Subjects	No. of cases	*In vitro* uptake (U) of Cr^{51}	*In vivo* half-life (T) of Cr^{51}-RBC	Viability (V)
Euthyroid	9	80.0%	24.0 days	>10 days
Hypothyroid	11	54.5%	13.8 days	<10 days
Euthyroid recipients of Cr^{51}-labeled blood from hypothyroid patients	5	—	12.8 days	<10 days
Hypothyroid patients treated with triiodothyronine	1	78.0%	—	—
	1	—	—	—

enced by a normal endocrine environment, as shown by the results of turnover studies on Cr^{51}-labeled blood from hypothyroid patients transferred into normal recipients. Apparently an inborn corpuscular defect is involved. It is a reversible trait that may be corrected by administration of active thyroid hormone, such as 3,5,3′-triiodothyronine. The corrective action appears to be mediated through the bone marrow.

An erythropoietic function of the thyroid gland, exerting its effects by way of the bone marrow, has been suggested for many years by experimental and clinical work (10–12). Deficiency of this erythropoietic function would explain the anemia of myxedema. The nature of the deficiency could be decreased cellular production (13) and/or arrest of erythroblast maturation (14). Either of these conditions would result in fewer red cells reaching the circulation. Since these red cells last a shorter time in hypothyroidism, the frequency of anemia is understandable.

If we assume that the thyroid gland has a hematopoietic function, then we can speculate whether the effects are those of response by a target tissue to a direct stimulus, or whether they are indirect manifestations of an adaptation to variable blood oxygen tension. Decreased body tissue requirements for oxygen in hypothyroidism can conceivably lead to a decrease in erythropoietic tissue in the bone marrow, but it could hardly explain a hypoplasia of the leukopoietic or megakariocytic series. The bone marrow of human beings subjected to the low oxygen tension of high altitudes exhibits a compensatory hyperplasia limited exclusively to the erythropoietic tissue (15). A direct effect of the thyroid on the marrow seems more plausible. In thyroidectomized animals there is no erythrocytic re-

sponse to lowered blood oxygen tension (16). All myeloid elements of the human marrow show increased activity during stimulation with thyroid hormone. The remarkable change to normal values for erythrocytic Cr^{51} uptake in 2 of our treated cases of hypothyroidism indicates a reversal to normal life span of the red cell under the influence of thyroid hormone therapy.

REFERENCES

1. Axelrod, A. R., and Berman, L.: The bone marrow in hyperthyroidism and hypothyroidism, *Blood* **6**: 436, 1951.
2. Ashby, W.: Determination of length of life of transfused blood corpuscles in man, *J. Exper. Med.* **29**: 267, 1919.
3. Gray, S. J., and Sterling, K.: Tagging of red cells and plasma protein with radioactive chromium, *J. Clin. Invest.* **29**: 1604, 1950.
4. Ebaugh, F. G., Jr.; Emerson, C. P., and Ross, J. F.: Use of radioactive chromium 51 as erythrocyte tagging agent for determination of red cell survival in vivo, *J. Clin. Invest.* **32**: 1260, 1953.
5. Necheles, T. F.; Weinstein, I. M., and LeRoy, G. V.: Radioactive sodium chromate for study of survival of red blood cells, *J. Lab. & Clin. Med.* **42**: 358, 1953.
6. Mollison, P. L., and Veall, N.: The use of the isotope 51Cr as a label for red cells, *Brit. J. Haematol.* **1**: 62, 1955.
7. Read, R. C.: Studies of red cell volume and turn-over using radiochromium, *New England J. Med.* **250**: 1021, 1954.
8. Read, R. C.; Wilson, G. W., and Gardner, F. H.: The use of radioactive sodium chromate to evaluate the life span of the red blood cell in health and certain hematologic disorders, *Am. J. M. Sc.* **228**: 1, 1954.
9. Busó, R.; Olavarrieta, S. T.; Suárez, R. M.; Sabater, J., and Suárez, R. M., Jr.: Radiochromium uptake in vitro as a measure of red cell survival in vivo. Proceedings of 6th Congress, Internat. Soc. of Hematology, Boston, Mass., Aug. 27–Sept. 1, 1956 (in press).
10. Gordon, A. S.; Kado, P. C.; Finkelstein, G., and Charipper, H. A.: The thyroid and blood degeneration in the rat, *Am. J. M. Sc.* **212**: 385, 1946.
11. Lim, R. K. S.; Sarkar, B. B., and Graham Brown, J. P. H.: The effect of thyroid feeding on the bone marrow of rabbits, *J. Path. & Bact.* **25**: 228, 1922.
12. Villaret, M.; Justin-Besancon, L., and Royer, P.: L'anémie des myxoèdemateux, *Semaine hôp. Paris* **21**: 1435, 1938.
13. Bomford, R.: Anemia in myxedema; and the role of the thyroid gland in erythropoiesis, *Quart. J. Med.* N. S. **7**: 495, 1938.
14. Castle, W. B., and Minot, G. R.: Pathological Physiology and Clinical Description of the Anemias. New York, Oxford University Press, 1936.
15. Merino, C. F.: Studies on blood formation and destruction in the polycythemia of high altitude, *Blood* **5**: 1, 1950.
16. Meyer, O. O.; Thewlis, E. W., and Rusch, H. P.: The hypophysis and hemopoiesis, *Endocrinology* **27**: 932, 1940.

EFFECT OF TRIIODOTHYRONINE ON THE SERUM LIPIDS AND LIPOPROTEINS OF EUTHYROID AND HYPERTHYROID SUBJECTS*

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ABSTRACT

l-Triiodothyronine (0.4 to 6.0 mg. daily) was administered in fractional doses intravenously for three to twenty-three days to 3 euthyroid subjects, 1 hyperthyroid patient during a partial spontaneous remission, and 1 patient with severe thyrotoxicosis. Hypermetabolism was produced in the euthyroid subjects and increased in the hyperthyroid patients.

Serum lipid and lipoprotein levels began to decrease within one to four days after the beginning of triiodothyronine therapy. The decrease in total lipid varied from 15 to 50 per cent, in lipid phosphorus from 19 to 56 per cent, and in total cholesterol from 23 to 56 per cent. Changes in protein fractions were inconstant. The serum lipid values returned to the control levels within seven to fifteen days after triiodothyronine was omitted. The observed fall in lipid values persisted after cessation of therapy longer than did the other clinical and laboratory evidences of hypermetabolism, which subsided within four to six days.

THE inverse relationship between the level of circulating thyroid hormone and the level of serum cholesterol is well established (1–3). The availability of synthetic *l*-triiodothyronine[1] provided an opportunity for controlled studies of the effects of this analogue of thyroxine on serum lipid and lipoprotein partition in euthyroid and hyperthyroid subjects.

MATERIALS AND METHODS

The subjects for the study were 2 euthyroid men aged 31 and 36 years; 1 euthyroid woman aged 36; 1 hyperthyroid man aged 45, in a state of partial clinical remission; and 1 markedly hyperthyroid man, aged 36. All 5 patients were maintained on a metabolic-ward regimen with fixed food and fluid intakes, providing either 2,000 or 2,400 calories. Metabolic balances for sodium, potassium, chloride, nitrogen, phosphorus, calcium and

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[1] Synthetic *l*-triiodothyronine was generously supplied by Dr. J. Kapp Clark of Smith, Kline and French Laboratories.

water were determined by standard methods (4). Determinations of serum total lipid, lipid phosphorus and total cholesterol levels, and serum protein and lipoprotein partitions by paper electrophoresis, were made by methods previously described (5). Serum β-lipoprotein on oil red-O-stained filter paper strips was measured quantitatively by planimetry after scanning with a Photovolt No. 52-C densitometer using a 540-mμ filter. In Subject #1, the protein fractions on the naphthalene black-stained paper strips were similarly analyzed. In the other 4 patients, the protein-stained strips were cut into the individual fractions, the dye was eluted with 0.2 *N* NaOH, and the concentration in each fraction determined with a Fisher electrophotometer. From the resulting data and the chemically determined total protein values, the concentration of each serum protein fraction in grams was calculated.

Each subject received 0.4 to 6.0 mg. of *l*-triiodothyronine daily, administered intravenously in two or three fractional doses, for three to twenty-three days. Subject #5 (in whose serum only cholesterol was determined) received a single dose of 0.8 mg. of *l*-triiodothyronine seven days after cessation of a three-day course of therapy.

RESULTS

In the euthyroid subjects, clinical signs of hyperthyroidism developed, including varying degrees of tachycardia, irritability, tremor, insomnia and hyperkinesis. Concurrently, basal oxygen consumption was increased and the 24-hour thyroidal uptake of radioiodine was markedly reduced. The metabolic evidence of increased catabolism has been reported elsewhere (6). In the hyperthyroid patients, there was an increase in the signs and symptoms of thyrotoxicosis. Euthyroid Subject #2, when given triiodothyronine, exhibited the least impressive clinical signs and symptoms and the smallest changes in catabolism and serum lipid partition.

Within one to four days after the beginning of triiodothyronine therapy, there was a decrease in the levels of serum lipids and lipoproteins (Figs. 1–5; Table 1). The decreases were greatest in the thyrotoxic patient who received the largest doses of triiodothyronine. Following cessation of therapy, the serum values were restored to the control levels in seven to fifteen days. In euthyroid Subject #2, lipid values subsequently rebounded above the control levels. The triiodothyronine-induced decreases were as follows:

Total lipid levels decreased 15 to 50 per cent. Except for the patient with thyrotoxicosis (Subject #4), in whom the total lipid level fell to 406 mg. per 100 ml., the minimal values remained within the normal range (600–1,000 mg. per 100 ml.).

Lipid phosphorus levels decreased 19 to 56 per cent. Only in Subject #4 did the lipid phosphorus concentration fall to a level (4.6 mg. per 100 ml.) below the normal range (6–11 mg. per 100 ml.) in this laboratory.

Total cholesterol levels fell 23 to 66 per cent. In Subject #4 the pre-treatment value of 223 mg. dropped to 75 mg. per 100 ml. during treatment. In only 1 euthyroid subject (#3) did the value fall to a level (118 mg. per 100 ml.) below the normal range (150–300 mg. per 100 ml.).

TABLE 1. EFFECT OF *l*-TRIIODOTHYRONINE ON SERUM β-LIPOPROTEIN FRACTIONS

Subject	Period	Day*	β-Lipoprotein (units)†	Maximum fall
1	Control		113	
	Triiodothy.	4	92	
	Post-triiodothy.	2	66	42%
	Post-triiodothy.	10	86	
	Post-triiodothy.	20	131	
2	Control		26	
	Control		18	
	Triiodothy.	4	29	
	Triiodothy.	6	16	Not signif.
	Post-triiodothy.	5	22	
	Post-triiodothy.	16	20	
3	Control			
	Control		98	
	Triiodothy.	2	61	
	Triiodothy.	4	56	43%
	Post-triiodothy.	6	97	
	Post-triiodothy.	19	103	
4	Control		70	
	Control		60	
	Triiodothy.	3	53	
	Triiodothy.	10	46	
	Triiodothy.	12	20	71%
	Triiodothy.	15	57	
	Post-triiodothy.	6	57	
	Post-triiodothy.	34	66	

* Day: During triiodothyronine period = days elapsed from onset of therapy.
Post-triiodothyronine period = days elapsed from cessation of therapy.
† One unit = .01 sq. in., as determined by planimetry.

β-Lipoprotein levels fell 42–43 per cent in 2 of the 3 euthyroid subjects (Table 1). In Subject #2 who demonstrated the least clinical, catabolic and serum lipid response to triiodothyronine, lipoprotein levels fluctuated and did not fall significantly. In the thyrotoxic patient (Subject #4), who received the largest doses of triiodothyronine, the serum β-lipoprotein concentration fell 71 per cent. In Subjects #3 and #4 the maximum fall occurred after four and twelve days of therapy; in Subject #1, it occurred two days following cessation of therapy.

Proteins. Protein fractionation revealed inconsistent changes following

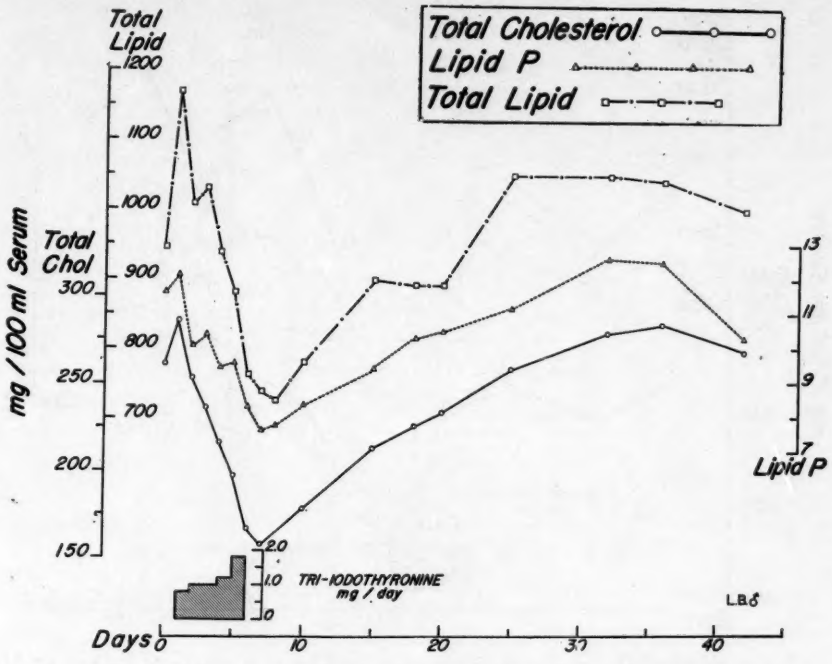

FIG. 1. Effect of *l*-triiodothyronine on serum lipid levels in euthyroid Subject #1.

administration of triiodothyronine. Therefore, the results could not be considered significant.

DISCUSSION

There are ample experimental and clinical demonstrations of the profound effect of thyroid hormone on blood lipids. Thus, when there is an excess of endogenous (7) or exogenous (8) thyroid hormone, blood lipid concentration is markedly diminished. Conversely, when thyroid hormone is deficient, as in spontaneous (9) and surgically (10) or medically (11) induced hypothyroidism, the blood lipid concentration is increased, and changes are also noted in the red cell:plasma ratios of cholesterol and lipid phosphorus (3, 12, 13).

More recently, it has been reported that administration of desiccated thyroid specifically lowers the serum concentration of low-density lipoproteins—the Sf 0–12 and Sf 12–20 fractions, as measured in the ultracentrifuge (14, 15).

Studies thus far reported on the effects of triiodothyronine on the serum lipids of man have been concerned with the decrease in cholesterol

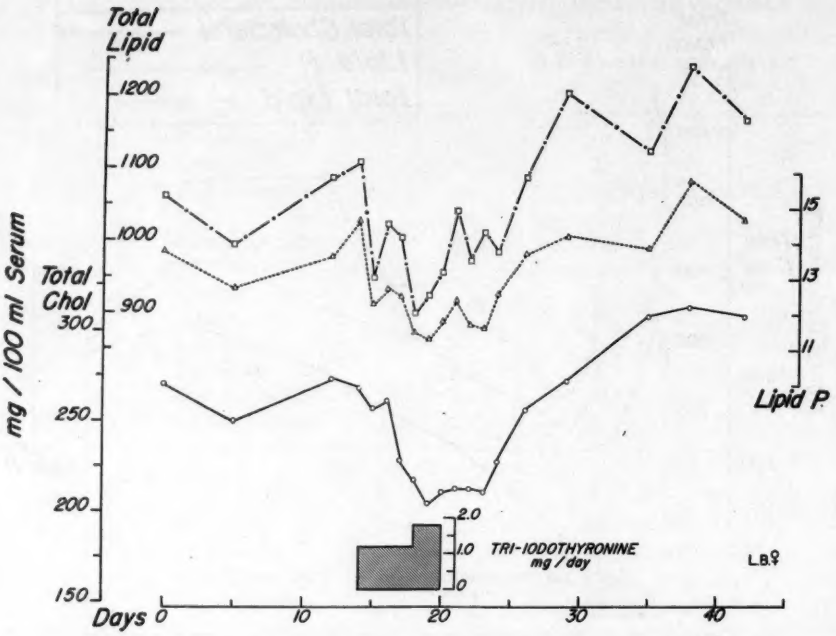

FIG. 2. Effect of *l*-triiodothyronine on serum lipid levels in euthyroid Subject #2.

concentration which is promptly induced in myxedematous patients by doses considerably lower than those of the present study (16–20). This decrease occurs as early as nine hours after administration (18), and the average fall is 8.6 per cent in the first twenty-four hours (20).

In 4 subjects with either postoperative or spontaneous myxedema, Asper *et al.* (16) observed decreasecl serum cholesterol levels lasting three, six, nine and twelve days after a single subcutaneous injection of 0.5 to 1.0 mg. of triiodothyronine. Maximum falls in cholesterol levels of 23, 29 and 47 per cent occurred following 1-mg. doses. In these subjects, single doses of *l*-thyroxine produced less rapid and smaller decreases in serum cholesterol concentration than did *l*-triiodothyronine (16).

The present series of euthyroid and hyperthyroid subjects initially had normal values for serum cholesterol and total lipid. All but Subject #2 had normal values for lipid phosphorus (in Subject #2 the control levels were elevated). Within one to four days, there was a decrease of all these constituents—most profound in the thyrotoxic subject who received the largest dose of triiodothyronine. In euthyroid Subjects #1 and #3 there were almost identical maximum percentage falls in the levels of total lipid, total cholesterol, and lipid phosphorus.

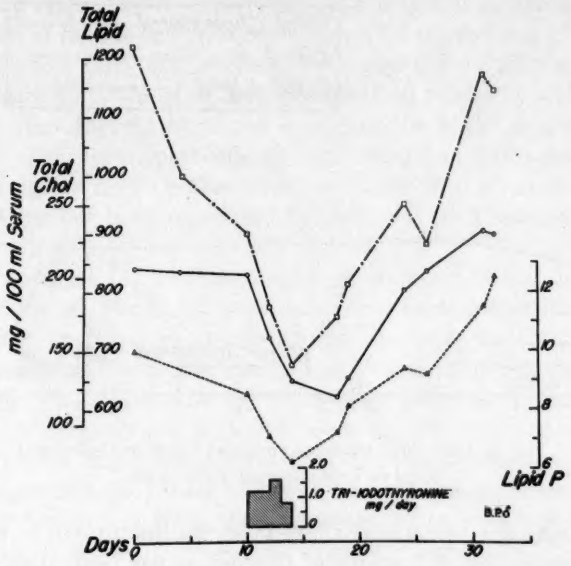

FIG. 3. Effect of *l*-triiodothyronine on serum lipid levels in euthyroid Subject #3.

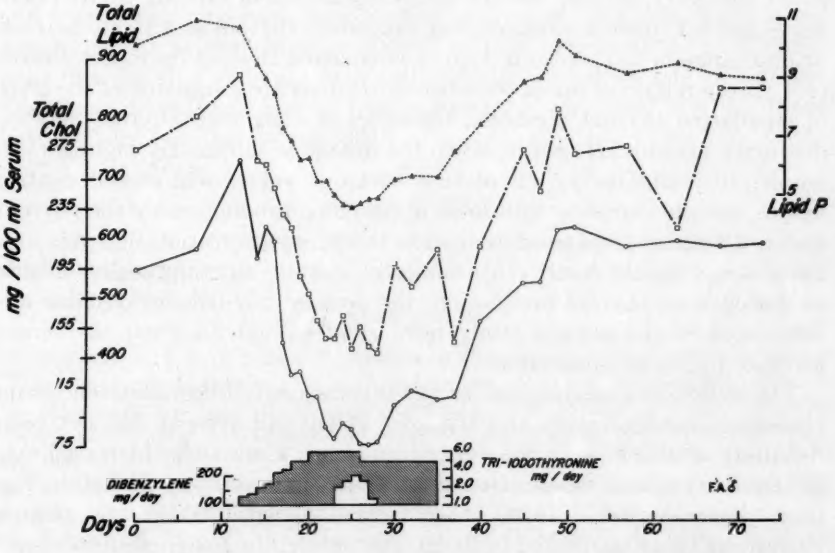

FIG. 4. Effect of *l*-triiodothyronine on serum lipid levels in hyperthyroid Patient #4.

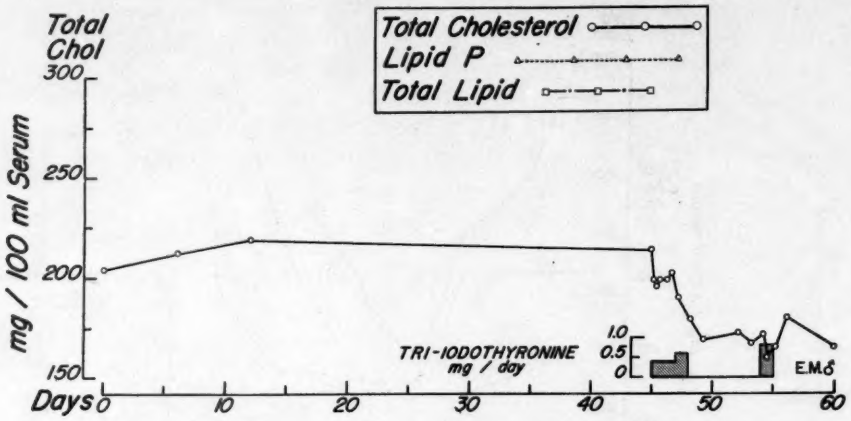

FIG. 5. Effect of *l*-triiodothyronine on serum cholesterol level in hyperthyroid Patient #5.

In explaining the variable changes in serum lipoprotein levels following daily administration of 3 grains of thyroid, it has been stressed that the administration of exogenous thyroid decreases the secretion of thyrotropin, which, in turn, causes lowered production and release of thyroid hormone (14). Inasmuch as the extent of this suppression of the thyroid gland may vary, as may the rate of disappearance of existing thyroid hormone, the net effect of administering exogenous thyroid may be an increase or no change in the concentration of circulating thyroid hormone. Therefore, if serum lipid or lipoprotein concentrations are a function of the level of circulating thyroid hormone, the effect of exogenous thyroid may be distinctly variable. However, when the dosage is sufficiently high (for example, 10 grains daily), circulating hormone values will exceed control values, despite complete shutdown of hormone production by the thyroid gland. Therefore, sustained reduction in the concentration of lipids and lipoproteins should result (15). Similarly, despite any suppressive effects on endogenous thyroid production, the dosages of *l*-triiodothyronine administered in the present study were great enough to lower the serum levels of lipid and lipoprotein.

The underlying mechanism for the inverse relationship between serum cholesterol concentration and the level of thyroid activity has not been definitely established. In the hyperthyroid rat, a markedly increased rate of hepatic synthesis, destruction and intestinal excretion of cholesterol has been demonstrated (21–24), the decreased concentration of plasma cholesterol being attributed to the greater acceleration of cholesterol excretion. The converse occurs in the hypothyroid state. According to this inter-

pretation, the major factor underlying the rise or fall in blood cholesterol concentration in hyper- or hypothyroidism is an altered rate of cholesterol turnover in which bile-acid (cholate) metabolism may play a role. More recent studies with C^{14}-labeled cholesterol in hyper- and hypothyroid mice have shown that thyroid hormone stimulates both the degradation and excretion of cholesterol, particularly with regard to the acidic products formed. The latter may be bile acids, since the formation of cholic acid from cholesterol has been reported (25), but may also include other fecal acidic products (26).

The demonstrated fall in cholesterol concentration in the clinical or experimental state is not related to the increased basal metabolic rate *per se* (3, 10, 12, 27). Increases in metabolic rate due to disorders unrelated to thyroid disease, or due to administration of dinitrophenol, do not affect the plasma cholesterol concentration (28); and increases in metabolic rate secondary to administration of thyroid do not cause lowering of the serum cholesterol level in nephrotic patients (29). In this connection it is significant that negative balances for nitrogen, phosphorus, potassium, calcium, sodium and chloride revert to control levels and may even become positive within four to six days after cessation of triiodothyronine therapy (6). On the other hand, in the present study, the observed falls in serum lipid and lipoprotein levels persisted for seven to fifteen days—considerably longer than did the clinical and laboratory evidences of increased metabolism.

More recently, the acetic-acid and propionic-acid analogues of thyroxine and triiodothyronine have been studied because of their capacity to lower serum cholesterol levels in some patients without increasing the basal metabolic rate (30, 31). Whether such a dissociation actually exists, or whether the effects differ from those of triiodothyronine when used in small doses, has not been clarified as yet (32).

Acknowledgment

We are indebted to Dr. Louis Leiter, Chief of the Medical Division, Montefiore Hospital, for helpful advice.

REFERENCES

1. Epstein, A. A., and Lande, H.: Studies on blood lipoids. I. The relation of cholesterol and protein deficiency to basal metabolism, *Arch. Int. Med.* **30**: 562, 1922.
2. Mason, R. L.; Hunt, H. M., and Hurxthal, L. M.: Blood cholesterol values in hyperthyroidism and hypothyroidism—their significance, *New England J. Med.* **203**: 1273, 1930.
3. Peters, J. P., and Man, E. B.: Interrelations of serum lipids in patients with thyroid disease, *J. Clin. Invest.* **22**: 715, 1943.
4. Weston, R. E.; Grossman, J.; Borun, E. R.; Guerin, H. A.; Mark, H.; Ullmann, T. D.; Wolfman, M., and Leiter, L.: Metabolic studies on the effects of ion

exchange resins in edematous patients with cardiac and renal disease, *Am. J. Med.* **14**: 404, 1953.

5. Sachs, B. A.; Cady, P., and Ross, G.: An abnormal lipid-like material and carbohydrate in the sera of patients with multiple myeloma, *Am. J. Med.* **17**: 662, 1954.
6. Isaacs, M. C.; Horowitz, H. B.; Sachs, B. A.; Essig, A., and Weston, R. E.: Metabolic effects of intravenously administered triiodothyronine in euthyroid human subjects, *Fed. Proc.* **15**: 99, 1956.
7. Nicholls, E. G., and Perlzweig, W. A.: Plasma fats and iodine absorption capacity of fatty acids in hyperthyroidism, *J. Clin. Invest.* **5**: 195, 1928.
8. Schmidt, L. H., and Hughes, H. B.: The free and total cholesterol content of whole blood and plasma as related to experimental variations in thyroid activity, *Endocrinology* **22**: 474, 1938.
9. Heckscher, H.: The fat-cholesterin content of the blood of cretins, *Biochem. Ztschr.* **158**: 422, 1925.
10. Boyd, E. M., and Connell, W. F.: Thyroid disease and blood lipids, *Quart. J. Med.* n.s. **5**: 455, 1936.
11. Handler, P.: Influence of thyroidectomy on liver and plasma lipides of choline and cystine deficient rats, *J. Biol. Chem.* **173**: 295, 1948.
12. Foldes, F. F., and Murphy, A. J.: Distribution of cholesterol, cholesterol esters and phospholipid phosphorus (a) in normal blood, *Proc. Soc. Exper. Biol. & Med.* **62**: 215, 1946; (b) in blood in thyroid disease, *Ibid.*, p. 218.
13. Schwarz, H., and Topper, A.: Cholesterol partitions of blood in myxedema (cretinism), *J. Pediat.* **3**: 242, 1933.
14. Strisower, B.; Gofman, J. W.; Galioni, E. F.; Almado, A. A., and Simon, A.: The effect of thyroid extract on serum lipoproteins and serum cholesterol, *Metabolism* **3**: 218, 1954.
15. Strisower, B.; Gofman, J. W.; Galioni, E.; Rubinger, J. H.; O'Brien, G. W., and Simon, A.: Effect of long-term administration of desiccated thyroid on serum lipoprotein and cholesterol levels, *J. Clin. Endocrinol. & Metab.* **15**: 73, 1955.
16. Asper, S. P., Jr.; Selenkow, H. A., and Plamondon, C. A.: A comparison of the metabolic activities of 3,5,3′-L-triiodothyronine and L-thyroxine in myxedema, *Bull. Johns Hopkins Hosp.* **93**: 164, 1953.
17. Selenkow, H. A., and Asper, S. P., Jr.: The effectiveness of triiodothyronine or thyroxine administered orally in the treatment of myxedema, *J. Clin. Endocrinol. & Metab.* **15**: 285, 1955.
18. Dyson, A., and Wood, M. W.: Triiodothyronine in myxoedema coma, *Lancet* **2**: 757, 1956.
19. De Gennes, L.; Deltour, G., and Leprat, V.: The effect of L-3,5,3′-triiodothyronine in ten cases of thyroid insufficiency, *Presse méd.* **61**: 1119, 1953.
20. Frawley, T. F.; McClintock, J. C.; Beebe, R. T., and Marthy, G. L.: Metabolic and therapeutic effects of triiodothyronine, *J.A.M.A.* **160**: 646, 1956.
21. Rosenman, R. H.; Byers, S. O., and Friedman, M.: The mechanism responsible for the altered blood cholesterol content in deranged thyroid states, *J. Clin. Endocrinol. & Metab.* **12**: 1287, 1952.
22. Rosenman, R. H.; Friedman, M., and Byers, S. O.: Observations concerning the metabolism of cholesterol in the hypo- and hyperthyroid rat, *Circulation* **5**: 657, 1952.
23. Friedman, M.; Byers, S. O., and Rosenman, R. H.: Changes in excretion of intesti-

nal cholesterol and sterol digitonides in hyper- and hypothyroidism, *Circulation* **5**: 657, 1952.

24. Weiss, S. B., and Marx, W.: The fate of radioactive cholesterol in mice with modified thyroid activities, *J. Biol. Chem.* **213**: 349, 1955.
25. Bloch, K.; Berg, B. N., and Rittenberg, D.: The biological conversion of cholesterol to cholic acid, *J. Biol. Chem.* **149**: 511, 1943.
26. Cook, R. P.; Polgar, N., and Thomson, R. O.: Cholesterol metabolism. 2. Cholesterol metabolism in the rat, *Biochem. J.* **47**: 600, 1950.
27. Peters, J. P., and Man, E. B.: The significance of serum cholesterol in thyroid disease, *J. Clin. Invest.* **29**: 1, 1950.
28. Cutting, W. C.; Rytand, D. A., and Tainter, M. L.: Relationship between blood cholesterol and increased metabolism from dinitrophenol and thyroid, *J. Clin. Invest.* **13**: 547, 1934.
29. Page, I. H., and Farr, L. E.: The influence of high and low fat diets and thyroid substance on plasma lipids of nephrotic patients, *J. Clin. Invest.* **15**: 181; 1936.
30. Trotter, W. R.: Effect of triiodothyroacetic acid on blood cholesterol levels, *Lancet* **1**: 885, 1956.
31. Oliver, M. F., and Boyd, G. S.: The influence of triiodothyroacetic acid on the circulating lipids and lipoproteins in euthyroid men with coronary disease, *Lancet* **1**: 124, 1957.
32. Gross, J.: Analogues of the Thyroid Hormone. Symposium. State University of New York, College of Medicine at New York City, June 3, 1957.

BIOLOGIC HALF-LIFE OF I^{131} IN THE THYROID OF HEALTHY MALES*

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ABSTRACT

The biologic half-life of I^{131} in the thyroid gland was measured in 38 healthy males ranging in age from 22 to 83 years. In subjects 50 years of age and over the biologic half-life of thyroidal I^{131} was directly proportional to the age of the subject and inversely proportional to the initial 24-hour percentage uptake in the gland. In subjects less than 50 years of age the biologic half-life was more or less independent of the age of the subject and of the 24-hour percentage uptake of I^{131}. Variation in the re-accumulation of I^{131} may explain this difference between young and old subjects. The results were compatible with the concept of a progressive decrease in secretion of thyroid hormone due to decreased peripheral utilization with advancing age.

IN A previous communication (1) it was reported that there is a progressive decrease in the 24-hour percentage uptake of I^{131} by the thyroid gland with advancing age. This decrease was thought to be clinically significant, in accord with the concept of decreased peripheral utilization of thyroid hormone in older persons (2). In the present study, further understanding of thyroid function in old age was sought by measuring the biologic half-life of thyroidal I^{131} following oral administration of a tracer dose (3).

SUBJECTS AND METHODS

The subjects were 38 male veterans ranging in age from 22 to 83 years. All were active, in good health and considered to be euthyroid by the usual clinical and laboratory criteria. None had received any medication likely to affect thyroid activity within one year prior to this study. All were selected from the same general population, ate the same food, and were either residents of or lived in the vicinity of Senneville Lodge, a domiciliary-care unit for veterans attached to Ste. Anne's Hospital.

A tracer dose of 50 microcuries of I^{131} was given orally to each subject and the percentage in the neck was measured twenty-four hours later and at frequent intervals thereafter, until counts were too low to be statistically reliable. Radioactivity in the thigh was counted 5 inches above the upper edge of the patella, until the values approxi-

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† Dr. J. C. Meakins, Director.

mated the background count. In some cases the first count was not made until seventy-two hours after the tracer dose of I^{131} was given.

The percentage of the tracer dose of I^{131} remaining in the thyroid gland each day was calculated as follows:

$$\frac{(\text{neck count} - (\text{background or thigh count})) \times \text{physical decay factor}}{\text{administered dose}}$$

When the thyroidal I^{131} uptake for each day was plotted on ordinary graph paper, the points for any given subject appeared to form a simple curve. When the data were plotted semi-logarithmically, the points for any given subject formed a straight line. In each case the "line of best fit" for all the points was calculated by the method of least squares, and for this purpose the I^{131} in the thyroid gland at twenty-four hours and at forty-eight hours was omitted, to allow for equilibration of the tracer dose in the body (Fig. 1). The day on which half of the theoretical 24-hour percentage uptake was still present in the neck, *i.e.*, biologic half-life, was derived from these data.

RESULTS AND DISCUSSION

There was a wide range in the biologic half-life of thyroidal I^{131}, varying from a low of twenty-one days to a high of two hundred days. When biologic half-lives were plotted against age on ordinary graph paper (Fig. 2), inspection revealed an interesting phenomenon. In subjects 50 years of age and over, there appeared to be a direct proportional relationship between

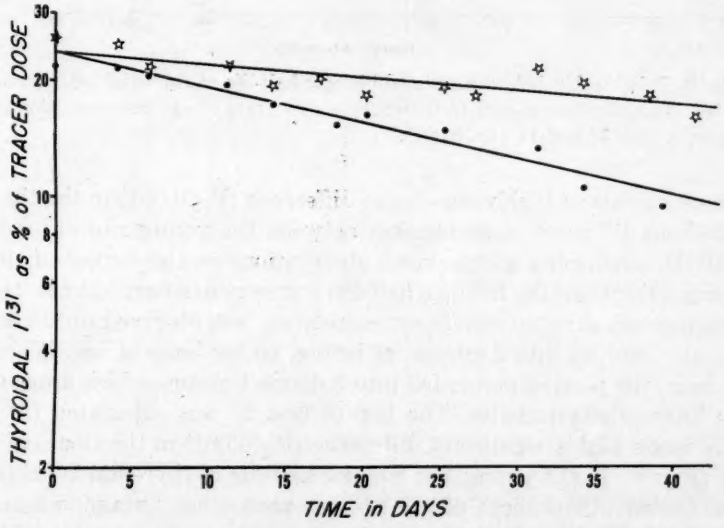

FIG. 1. Decay of thyroidal I^{131} in a young and an old subject after a single tracer dose. Stars = Subject D.M., aged 39; biologic half-life of I^{131}, 88 days. Dots = Subject A.L., aged 69; biologic half-life of I^{131}, 36 days. Day zero is 72 hours after administration of the tracer dose.

age and biologic half-life of I^{131}, whereas in subjects less than 50 years of age there was no such correlation. Indeed, in the latter, the half-life appeared to decrease slightly with age.

The data were accordingly divided into two groups: 1) in subjects less than 50 years of age, and 2) in subjects 50 years of age and over. The line of best fit for each group was calculated. There was a highly significant difference ($P < 0.001$) (4) in the slopes of these lines (Fig. 2), although there was no significant difference ($0.2 > P > 0.1$) in the biologic half-lives themselves (Table 1).

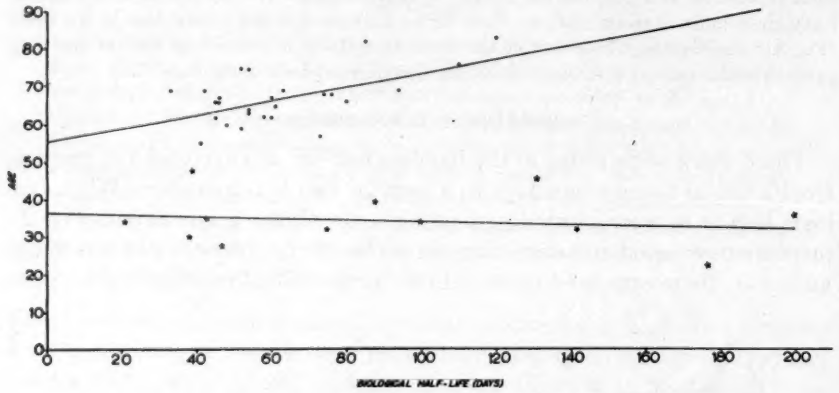

FIG. 2. Relationship between age and biologic half-life of thyroidal I^{131}. Stars = Subjects less than 50 years of age. Dots = Subjects 50 years of age and over. Comparison of slopes of lines of best fit ($P < 0.001$).

There was also a highly significant difference ($P < 0.001$) in the theoretical 24-hour I^{131} percentage uptakes between the young and old subjects (Table 1), confirming our previous observations on this aspect of thyroid function (1). When the biologic half-lives were plotted against the 24-hour percentage uptakes, no significant correlation was observed until the subjects were divided into 2 groups, as before, on the basis of age. When this was done, the plotted points fell into 2 distinct groups which appeared to have linear characteristics. The line of best fit was calculated for each. There was a highly significant difference ($P < 0.001$) in the slopes of these lines (Fig. 3). In the young, the biologic half-life of thyroidal I^{131} appeared to be almost independent of the 24-hour percentage uptake; whereas, in the old, the biologic half-life was inversely proportional to the 24-hour percentage uptake.

The day-to-day level of I^{131} in the thyroid gland, from which the biologic half-life is calculated, depends upon the rate of secretion and the rate

TABLE 1. RELATIONSHIPS BETWEEN 24-HOUR THYROIDAL I^{131} UPTAKE, BIOLOGIC HALF-LIFE, AND AGE OF SUBJECT

Age of subject (yrs.)		Thyroidal I^{131} uptake at 24 hrs. (%)		Biologic half-life of thyroidal I^{131} (days)	
Young	Old	Young	Old	Young	Old
22	55	32.6	19.7	176.7	40.6
28	57	28.0	24.4	47.0	73.3
30	59	22.0	20.3	37.7	52.3
32	60	32.9	22.8	74.9	47.7
32	62	20.5	17.0	141.9	45.1
34	62	29.0	26.8	21.0	57.9
34	62	24.3	22.4	99.5	60.4
35	63	35.0	30.8	43.0	53.8
35	64	24.9	15.4	200.3	53.5
39	65	23.5	12.8	88.0	44.7
46	65	24.4	22.9	131.4	61.3
48	66	24.0	12.3	39.1	79.5
	66		22.0		46.0
	67		19.3		46.0
	67		25.5		60.2
	68		17.9		75.7
	68		16.3		74.1
	69		23.4		33.1
	69		18.5		62.6
	69		23.1		93.8
	69		13.2		41.8
	75		14.5		51.7
	75		22.9		54.4
	76		11.7		110.2
	82		16.8		86.0
	83		8.2		119.6
Mean ±S.E.					
35	67	26.8	19.2	91.7	62.5
		1.4	1.0	17.1	4.2
			$P<0.001$		$0.2>P>0.1$

of re-accumulation by the gland (5, 6). For the first few days re-accumulation of I^{131} plays a negligible role, but this factor is of greater significance in later measurements (7). When re-accumulation of I^{131} is prevented by the use of blocking agents, such as methimazole, the biologic half-life may be correlated with the secretion rate in the gland. This technique has been

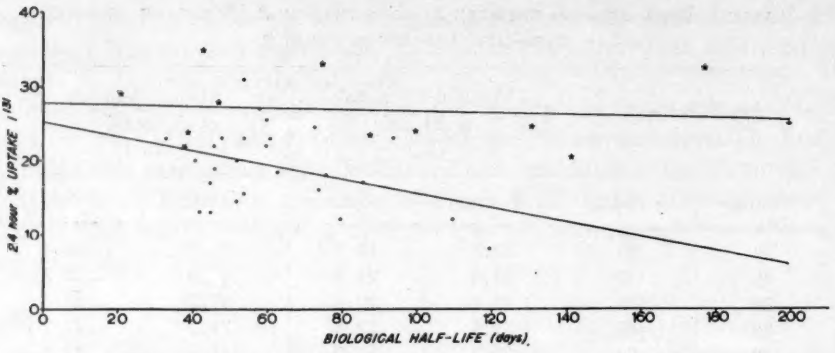

FIG. 3. Relationship between 24-hour percentage uptake of I^{131} and biologic half-life in the thyroid. Stars = Subjects less than 50 years of age. Dots = Subjects 50 years of age and over. Comparison of slopes of lines of best fit ($P < 0.001$).

extensively used to study the effect of various substances on the secretion rate (8–13).

Since re-accumulation of I^{131} was not prevented in the present study, no definite conclusions about the secretion rate in the thyroid gland can be made on the basis of our data. The biologic half-life was inversely proportional to the 24-hour percentage uptake in the older subjects, although the results showed a wide scatter. One might speculate, therefore, that re-accumulation of I^{131} made available from peripherally metabolized thyroid hormone is of little importance in the aged, perhaps due to increased renal excretion. Another possibility is that the end-product of peripherally metabolized hormone differs between young and old subjects, and that in the latter the I^{131} is not as readily available for re-accumulation by the thyroid gland.

The relationship of the 24-hour percentage I^{131} uptake to the secretion rate of the thyroid and to the biologic half-life has not been extensively studied in the aged. For example, in Greer's paper (12) only 6 of the 20 euthyroid subjects studied were more than 50 years of age, and no mention was made of the 24-hour percentage uptake of I^{131}. The present data suggest that with advancing age there is a progressive decrease in the 24-hour percentage uptake of I^{131} by the thyroid gland, coupled with a diminishing secretion rate by the gland—compatible with the concept of decreased peripheral utilization of thyroid hormone. This concept is supported by the finding of normal levels of serum protein-bound iodine which were not significantly different between young and old subjects (data not shown in this article). Studies using blocking agents, such as methimazole, would help to clarify these relationships, as well as the relative importance of re-accumulation in the young versus the aged.

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REFERENCES

1. ROSENBERG, G.: Effect of age on 24 hour I-131 uptake in healthy males, *Canad. Services M. J.* **13**: 565, 1957.
2. PERLMUTTER, M., and RIGGS, D. S.: Thyroid collection of radioactive iodide and serum protein-bound iodine concentration in senescence, in hypothyroidism and in hypopituitarism, *J. Clin. Endocrinol.* **9**: 430, 1949.
3. BURNS, F. J.; FISH, W. A.; HACKETT, J. W., and HICKEY, F. C.: Radioactive decay and metabolic loss of iodine from normal thyroid, *J. Applied Physiol.* **4**: 15, 1951.
4. EISENHART, C., and WILSON, P. W.: Statistical methods and control in bacteriology, *Bacteriol. Rev.* **7**: 57, 1943.
5. RIGGS, D. S.: Quantitative aspects of iodine metabolism in man, *Pharmacol. Rev.* **4**: 284, 1952.
6. STANBURY, J. B.: Synthesis, storage, and release of thyroid hormone, *in* The Thyroid, ed. by S. C. Werner. New York, Paul B. Hoeber Inc., 1955, p. 10.
7. RIGGS, D. S.: Quantitative aspects of thyroid hormone turnover, *in* The Thyroid, ed. by S. C. Werner. New York, Paul B. Hoeber, Inc., 1955, p. 41.
8. GOLDSMITH, R. E.; STANBURY, J. B., and BROWNELL, G. L.: The effect of thyrotropin on the release of hormone from the human thyroid, *J. Clin. Endocrinol.* **11**: 1079, 1951.
9. SOLOMON, D. H.: Studies of the fractional rate of release of hormonal iodine from the thyroid gland, (abstract) *J. Clin. Endocrinol. & Metab.* **14**: 772, 1954.
10. SOLOMON, D. H.: Factors affecting the fractional rate or release of radioiodine from the thyroid gland in man, *Metabolism* **5**: 667, 1956.
11. GREER, M. A., and DEGROOT, L. J.: A study of the effect of stable iodide on thyroid secretion in man, *Metabolism* **5**: 682, 1956.
12. GOLDSMITH, R. E., and EISELE, M. L.: The effect of iodide on the release of thyroid hormone in hyperthyroidism, *J. Clin. Endocrinol. & Metab.* **16**: 130, 1956.
13. GREER, M. A., and SHULL, H. F.: A quantitative study of the effect of thyrotropin upon the thyroidal secretion rate in euthyroid and thyrotoxic subjects, *J. Clin. Endocrinol. & Metab.* **17**: 1030, 1957.

COMPARATIVE STUDY OF THE 17-KETOGENIC (NORYMBERSKI), GLENN-NELSON AND REDDY METHODS FOR THE DETERMINATION OF C_{21} URINARY STEROIDS

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ABSTRACT

A series of urine samples was analyzed for 17-OH-corticosteroids by the 17-ketogenic steroid method of Norymberski, the Glenn-Nelson method and the Reddy technique. The results indicate relatively good correlation between the Norymberski and Glenn-Nelson values and the clinical findings, but serious discrepancies were obtained with the Reddy method, especially in urines containing only small amounts of corticosteroids. Utilizing 3 wavelengths for reading with the latter procedure, although frequently altering the values, did not necessarily improve their agreement with those obtained by the other 2 methods nor with the final diagnosis. Data are presented which indicate that the Glenn-Nelson technique leaves a significant percentage of the 17-OH-corticosteroids in the residual urine following hydrolysis and extraction.

The results point up the advantage of the Norymberski procedure in the diagnosis of the adrenogenital syndrome, a condition in which pregnanetriol is excreted in abnormal amounts and measured as a 17-ketogenic steroid.

IN RECENT years the determination of C_{21} urinary corticosteroids has assumed great importance as an aid in the diagnosis and treatment of adrenal disease. The wide choice of analytical methods available has posed questions as to the specificity and quantitative reliability of these methods, since they do not, in all cases, yield comparable results. The authors have selected 3 of these procedures for a comparative study—the 17-ketogenic steroid method of Norymberski, and the methods of Glenn-Nelson and of Reddy which employ the Porter-Silber color reaction. Previous comparisons of Porter-Silber methods and the 17-ketogenic steroid procedure have recently been reported (1, 2, 3).

METHODS

17-Ketosteroids (17-KS) and 17-ketogenic steroids (17-KGS) were determined as described in a previous study (4). The technique for determination of pregnanetriol in selected cases was that of Cox (5).

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The Glenn-Nelson (G-N) method (6) was used with one modification: the steroid conjugates were first extracted from urine by the procedure of Edwards, Kellie and Wade (7) and then treated with glucuronidase. This step was introduced because the authors have confirmed the belief (8) that some urines contain substances which appear to inhibit glucuronidase; in such cases, prior extraction of the steroid conjugates has occasionally yielded higher results than direct treatment of the urine specimen with enzyme.

For the determination of Porter-Silber chromogens by the Reddy method, the procedure described by Reddy (9) was used with 2 modifications: (a) The butanol was purified as follows: to a liter of *n*-butanol are added 2 grams of dinitrophenylhydrazine and 0.5 ml. of concentrated HCl. The mixture is allowed to stand in the dark for two days and is then distilled, the fraction distilling at 117° C. being collected. This fraction is redistilled. This purification procedure is simpler than that described by Reddy and yields a superior solvent. The absorbances of a reagent blank against water as reference solution were 0.018, 0.006 and 0.004 at 370, 410 and 450 mμ respectively, using butanol purified as described. The absorbances of a similar reagent blank made with butanol purified by Reddy's procedure were 0.096, 0.019 and 0.016 at 370, 410 and 450 mμ, respectively. The absorbances of the same butanol, before purification, were 0.335, 0.061 and 0.034 at the same 3 wavelengths. (b) The colors obtained in the Porter-Silber reaction were read and steroid excretion calculated as described in Reddy's procedure (9). Readings were also made, however, at 370, 410 and 450 mμ (10), and the "corrected" absorbances (calculated by the base-line technique (11)) were used to calculate the steroid excretion. In using the Porter-Silber reaction, colors were developed overnight at room temperature (approximately 25° C.).

RESULTS

Comparative results by three methods

The results from a representative group of urine samples analyzed by the 3 procedures for 17-OH-corticosteroids are shown in Table 1. In addition are listed the 17-ketosteroid values obtained on each sample and the pregnanetriol levels, when determined. The urine samples were selected from routine specimens submitted to the laboratory for analysis of 17-OH-corticosteroids. The diagnoses were made by the attending physicians on the basis of clinical and laboratory findings.

Normal values (mg. per twenty-four hours) for adults for the individual tests were considered to be as follows:

	Male	*Female*
17-ketosteroids	9–22	6–15
Pregnanetriol	0– 3	0– 3
17-OH-corticosteroids		
Norymberski (17-KGS)	9–25	6–18
Glenn-Nelson	3–10	2– 6
Reddy	1.1–10.7	1.1–10.7

The normal range for the Reddy method is apparently not yet well established, as indicated by marked variations in reported results (9, 10,

TABLE 1. COMPARISON OF ASSAYS OF URINE SAMPLES BY THE 17-KGS, GLENN-NELSON AND REDDY TECHNIQUES

Patient	Age & sex	17-KS (mg./24 hrs.)	17-OH-corticosteroids (mg./24 hrs.)				Pregnanetriol (mg./24 hrs.)	Diagnosis
			17-KGS	G-N	Reddy			
					Uncorrected*	Corrected†		
R.C.	43 yrs. F	0.8	2.6	1.6	5.4	20.0		Adrenocort. insufficiency
E.P.	45 yrs. F	0.4	1.0	0.5	1.9	1.5		Adrenocort. insufficiency
G.B.	43 yrs. F	0.9	6.3	7.0	10.5‡	11.0		Suspected adrenocort. insufficiency
M.H.	39 yrs. F	1.6	0.8	2.2	1.6‡	2.3		Hypopituitarism
E.S.	35 yrs. M	5.3	4.3	2.3	1.6	2.7		Hypopituitarism (pit. tumor)
R.S.	3 mo. M	0.05	0.6	0.6	1.7	<0		Normal infant
B.C.	8 da. M	0.06	0.2	0.2	0.4	0.5		Normal infant
G.H.	4 mo. M	0.7	5.2	1.2	14.7	17.4		Adrenogen. syn.
L.C.	5 mo. F	2.8	9.4	0.0	4.8	5.1	5.5	Adrenogen. syn.—before cortisone
L.C.		1.3	41.0	15.0	33.3	37.6	0.6	Adrenogen. syn.—after cortisone
J.W.	9 yrs. F	15.7	43.6	0.9	5.0	3.2	25.9	Adrenogen. syn.; pseudohermaph.
F.B.	40 yrs. F	24.0	49.8	1.4	8.0	6.3	36.8	Post-pubertal adrenogen. syndrome
F.B.		67.4	166.0	1.7	18.4	15.8	99.5	Post-ACTH
N.H.	36 yrs. F	14.0	19.2	7.8	16.7	19.8		Cushing's syn., adrenal hyperpl.—before ACTH
		31.8	72.2	57.2	114.0	124.0		Cushing's syn., adrenal hyperpl.—after ACTH
D.B.	40 yrs. M	4.1	44.9	17.0	48.9	22.0		Cushing's syn.—before ACTH
D.B.		6.5	109.5	26.0	68.5	75.0		Cushing's syn.—after ACTH
D.B.		4.3	58.2	16.9	39.0‡	53.3		Cushing's syn.—after ACTH
M.V.	2 yrs. F	0.4	2.3	1.8	4.2‡	0.9		Suspected Cushing's syn.
I.P.	Adult F	2.9	7.1	3.4	9.7	11.1		Basal hypoadrenocorticism—before ACTH
I.P.		6.8	45.7	14.5	35.5	40.0		Basal hypoadrenocorticism—after ACTH
L.L.	40 yrs. F	0	1.9	1.6	7.5	24.6		Basal hypoadrenocorticism—before ACTH
L.L.		1.7	14.3	11.0	20.4	39.6		1st day ACTH
L.L.		1.9	14.2	8.6	14.5	18.4		2nd day ACTH
E.C.	32 yrs. F	7.1	10.9	3.1	<0‡	14.5		Hypochromic anemia—before ACTH
E.C.		12.4	19.4	4.4	13.4	16.2		Hypochromic anemia—after ACTH

* Read at 410 mμ only; corrected for blank only.
† Read at 370, 410 and 450 mμ; corrected by base-line technique.
‡ Intense brown, violet or red colors present in final extracts.

12–14). The establishment of the lower level for adult normal subjects is especially important in any attempt to utilize this procedure for diagnosis of adrenocortical insufficiency. The range listed here as normal was that given by Reddy and associates (9).

In the 2 definitely established cases of adrenocortical insufficiency (Patients R.C. and E.P.), both the 17-KGS and G-N methods agreed well, in that the urinary 17-OH-corticosteroids were very low. The level of 17-ketosteroids also indicated a markedly depressed adrenal activity. In Patient R.C., the Reddy method indicated an adrenal steroid output that was well within the normal range but, when calculated by the base-line technique, was an abnormally elevated value. In Patient E.P., the Reddy values, although not below the normal range listed for this method, were decidedly at the low end of the range.

In the 2 cases of hypopituitarism (Patients M.H. and E.S.) there was relatively good agreement among the results of all 3 procedures for 17-OH-corticosteroids.

R.S., a normal infant, showed the expected trace amounts of 17-ketosteroids and 17-OH-corticosteroids by the 17-KGS and G-N procedures. By the Reddy technique, however, a value of 1.7 mg. per twenty-four hours—a high value for an infant—was obtained by the usual method of calculation. When the results were calculated on the basis of 3 wavelengths, a negative value was obtained.

In cases of adrenogenital syndrome, in which a metabolic abnormality in the formation of cortisone results in the formation and excretion of excessive amounts of pregnanetriol, it is expected that the 17-KGS and G-N or Reddy procedures will show divergent results. Pregnanetriol is measured as a 17-ketogenic steroid, but is not included as a Porter-Silber chromogen. This is illustrated in the cases of G.H. and L.C. (both infants), J.W. (a 9-year-old child) and F.B. (an adult). In our laboratories, 17-KGS values for normal infants under 1 year of age were less than 1 mg. per twenty-four hours in 5 cases, confirming results in 3 normal infants under the age of 1 year published by Borth *et al.* (15). In children under 10 years of age the values are normally less than 6 mg. per twenty-four hours (16, 17). The results in the 4 patients just mentioned are therefore definitely elevated. The G-N values for the same patients are, in our experience, either normal or low for their age groups. The Reddy results can only be interpreted as spurious.

Relatively equivalent results were obtained in both of the cases of Cushing's syndrome (Patients N.H. and D.B.) by all 3 methods.

In 2 cases which were classified as basal hypoadrenocorticism (Patients L.L. and I.P.) according to Prunty's definition (18), urinary 17-ketosteroids were low and 17-OH-corticosteroids were low or low-normal by both the 17-KGS and G-N techniques. The Reddy values, however, would indicate normal or high excretion of these compounds, depending on the method of calculation employed.

Efficiency of recovery by the Glenn-Nelson method

It is not expected that the 17-KGS and the G-N values should be identical when complete recovery is obtained by both methods. In the former case the measurement is based on colorimetric values of the liberated 17-ketosteroids, using DHA as a standard, and in the latter method the measurement is based on a standard of a C_{21}-compound, cortisol. In addition, the G-N method, employing the Porter-Silber color reaction, measures only corticosteroids having a 17,21-diol-20-one grouping, whereas the 17-KGS technique also includes 17,20-diol,21-methyl and 17,20,21-triol com-

pounds (16). If the direct Norymberski 17-KGS procedure with borohydride is used, an additional grouping, 21-methyl,17,20-ketol, is also measured. Nevertheless, the discrepancies between values obtained by the G-N and 17-KGS procedures frequently appear to be considerably greater than would be expected if both methods are efficient in recovery of the steroids to be measured. Since the 17-KGS results are usually higher than the G-N results on the same urines, it was felt advisable to check the efficiency of the enzyme hydrolytic procedure utilized in the G-N method.

Urine samples were assayed for 17-KGS by the direct method and by the G-N and Reddy methods as described. The enzymatic hydrolysis was carried out for 48 hours at 37° C. Following chloroform extraction, the residual urine of each sample was then assayed for corticosteroids by the 17-KGS and Reddy methods. Any corticosteroids remaining in this residual fraction represent those compounds either not hydrolyzed or not extracted by the G-N method. In addition, each chloroform extract was also assayed by the 17-KGS and Reddy methods without passing through the Florisil column used in the routine G-N procedure. Table 2 shows the results obtained. The Reddy values were calculated on the basis of the reading at 410 mμ only.

TABLE 2. EFFICIENCY OF THE GLUCURONIDASE HYDROLYSIS PROCEDURE AS DETERMINED BY ASSAYS FOR 17-OH-CORTICOSTEROIDS ON EXTRACTED URINE AND THE CHLOROFORM EXTRACT

Subject	Mg./24 hrs. found on direct analysis of urine			Mg./24 hrs. found in residual extracted urine		Mg./24 hrs. found in chloroform extract	
	[1] 17-KGS	[2] Reddy	[3] G-N	[4] 17-KGS	[5] Reddy	[6] 17-KGS	[7] Reddy
A	42.7	27.8	13.9	15.0 (35.1)*	8.2 (29.5)	26.7 (62.5)	11.7 (42.1)
B	22.7	26.0	10.4	6.0 (26.4)	7.9 (30.4)	17.9 (79.0)	11.9 (45.8)
C	51.0	32.9	24.8	19.7 (38.5)	8.5 (25.8)	35.4 (69.4)	20.6 (62.6)
D	30.7	17.3	8.8	12.3 (40.0)	5.1 (28.5)	18.0 (58.7)	8.0 (46.2)

* Figures in parentheses indicate the percentage of the original values (columns 1 and 2) found by direct analysis of the urine.

The fact that all Porter-Silber chromogens are not extracted from urine by the G-N technique is demonstrated in Table 2. Columns 4 and 5 in Table 2 show that 26.4 to 40.0 per cent of the original 17-KGS present are left behind in the extracted urine and, by the Reddy technique, from 25.8 to 30.4 per cent of Porter-Silber chromogens remain unextracted. Since it is well known that chloroform as used in the G-N procedure will extract free corticosteroids almost completely, it must be concluded that the hydrolytic procedure leaves behind a considerable amount of unhydrolyzed glucuronidates and/or other conjugates. Theoretically, the values in columns 4 and 6 should equal 100 per cent of those in column 1; as determined, they range from 97.6 to 107.9 per cent. By the Reddy method, the recoveries from the residual urine and extract (columns 5+7) were only 71.6 to 88.4 per cent of the original Reddy levels (column 2).

DISCUSSION

Urine is a solution of great chemical complexity and variability. Therefore, the specificity is an important factor in determining the usefulness of a diagnostic test; but achievement of the ultimate in specificity is an ideal situation seldom realized or actually seldom necessary. A crude test, simple to perform and having only moderate specificity may still be of considerable value. If, however, its lack of specificity is of sufficient magnitude and frequency as to mislead, then any advantage of the test from the standpoint of simplicity is more than nullified.

Although the G-N and the Reddy methods should theoretically measure the same compounds, the variable background material remaining at times in the crude Reddy extract may result in a serious lack of specificity as indicated, for example, by occasional high values obtained in normal children and Addisonian patients. Attempts to correct for background interference by utilizing a correction equation (11) are not necessarily helpful because the basic assumption regarding the absorbance of the background impurities is not always valid for these extracts. This is illustrated in the Patients R.C. and L.L. (Table 1) for whom the absorbances calculated by the base-line technique are higher than the "uncorrected" values. This can occur when the absorption curve for the urine blank, instead of lying on a straight line, is U-shaped, *i.e.*, the absorbance at 410 mμ is considerably less than at 370 mμ and 450 mμ. Applications of the base-line correction method must be restricted to those procedures in which it has been experimentally proved that the absorbances of background impurities lie on a straight line at the wavelengths chosen for analysis.

In certain urine extracts, the development of nonspecific colors with the phenylhydrazine reagent in the Reddy method makes it difficult to obtain valid readings at the proper wavelength. It has already been noted (1, 19,

20) that a number of therapeutic agents produce colors which interfere with this procedure.

Wolfson (1) compared results on a patient before and after administration of hydrocortisone, using the 17-ketogenic steroid procedure and a Port-Silber method. Both methods yielded comparable results. Moxham and Nabarro (2) compared a modified Reddy procedure and Norymberski's 17-ketogenic procedure on 400 urine specimens. In general a good correlation was obtained, although with some urines large differences were noted between the two procedures. Butt, Kornel and Morris (3) tested the urines of 5 patients at intervals before and after administration of ACTH, employing a modified Reddy method and the 17-ketogenic technique. They believed that good correlation was obtained in most samples. Little attention has apparently been paid to comparisons of the methods at low urinary levels of steroids.

In general, good clinical agreement was obtained in this study between the 17-KGS procedure and the G-N technique despite the fact that the former method measures certain steroids not measured by the Porter-Silber reaction. As seen in Table 1, values for 17-KGS include those for the 11-keto derivative of pregnanetriol in addition to pregnanetriol, both of which are present only in small amounts except in certain specific conditions. In our opinion, the inclusion of these compounds is a distinct advantage in revealing the presence of abnormal steroids which would pass unnoticed by the G-N technique or by any other method utilizing the Porter-Silber reagent. For example, the 17-KGS values in the cases of adrenogenital syndrome listed in Table 1 are dramatically elevated, whereas the G-N values are either normal or equivocal for the age groups represented. The Reddy results would be considered high in 2 of these cases, but the values obtained must obviously be due to fortuitous circumstances, since pregnanetriol is not measured by this procedure. In cases of adrenogenital syndrome in which both 17-KGS and pregnanetriol were determined, the results indicate that the latter compound often constitutes the major portion of the C_{21} compounds excreted.

The almost consistently higher values obtained by the 17-KGS and Reddy procedures as compared to the G-N method suggest that a variable percentage of the C_{21} steroids present are not being measured by the G-N technique. The enzymatic hydrolysis utilized in this procedure has been criticized (8) on the basis that it is limited to glucuronidates, whereas there is strong evidence that other conjugates are present (21, 22). In addition, urine samples vary in their susceptibility to this enzymic hydrolytic process and may contain inhibitors or competing substrates. The finding of a considerable percentage of the total steroids in the residual urine

(Table 2) is evidence that this method fails to recover quantitatively the available 17-OH-corticosteroids.

The Reddy procedure has the advantage of simplicity, apparently can be used to distinguish normal from abnormally high levels of corticosteroids, and may be satisfactory to evaluate the adrenocortical response to ACTH stimulation. In our experience the weakness of the method is its lack of specificity and resultant inaccuracy in the determination of low concentrations of corticosteroids as found, for example, in the urine of children and patients with hypoadrenalism.

The 17-KGS and Reddy methods recover more of the 17-OH-corticosteroids than does the G-N procedure, since they do not utilize enzymatic hydrolysis. The 17-KGS technique affords an additional advantage in measuring certain important steroids which are not included by any method using the Porter-Silber reagent. The 17-KGS method, although more complex than the Reddy method, is considerably less involved than the G-N procedure and fortunately lends itself admirably to the analysis of multiple samples.

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REFERENCES

1. Wolfson, W. Q.: Discussion *in* Recent Progress in Hormone Research, ed. by G. Pincus. New York, Academic Press Inc., 1954, vol. 9, pp. 290–296.
2. Moxham, A., and Nabarro, J. D. N.: Urinary glucocorticoid excretion, *J. Clin Path.* **9**: 351, 1956.
3. Butt, W. R.; Kornel, L., and Morris, R.: The determination of 17-OH corticosteroids in urine, *Acta endocrinol.* **26**: 65, 1957.
4. Sobel, C. S.; Golub, O. J.; Henry, R. J.; Jacobs, S. L., and Basu, G. K.: Study of the Norymberski methods for determination of 17-ketogenic steroids (17-hydroxycorticosteroids) in urine, *J. Clin. Endocrinol. & Metab.* **18**: 208, 1958.
5. Cox, R. I.: A method for the quantitative determination in urinary extracts of C_{21} 17:20-dihydroxy-20-methyl steroids, *Biochem. J.* **52**: 339, 1952.
6. Glenn, E. M., and Nelson, D. H.: Chemical method for the determination of 17-hydroxycorticosteroids and 17-ketosteroids in urine following hydrolysis with β-glucuronidase, *J. Clin. Endocrinol. & Metab.* **13**: 911, 1953.
7. Edwards, R. W. H.; Kellie, A. E., and Wade, A. P.: The extraction and oxidation of urinary steroid conjugates, *in* Memoirs of the Society for Endocrinology, No. 2, ed. by P. Eckstein and S. Zuckerman. London, Dennis Dobson Ltd., 1953, pp. 53–63.
8. Marrian, G. F.: The determination of steroids in blood and urine. Proc. 3rd Internat. Congress Biochemistry, Brussels, Belgium, 1955, pp. 511–515.
9. *a*) Reddy, W. J.: Modification of the Reddy-Jenkins-Thorn method for the estimation of 17-hydroxycorticoids in urine, *Metabolism* **3**: 489, 1954.

b) JENKINS, D.; FORSHAM, P. H.; LAIDLAW, J. C.; REDDY, W. J., and THORN, G. W.: Use of ACTH in the diagnosis of adrenal cortical insufficiency, *Am. J. Med.* **18:** 3, 1955.

10. HERTOGHE, J., and CRABBE, J.: Application de la correction de Allen à la détermination de l'excrétion urinaire des 17-hydroxycorticoïdes par la méthode de Reddy, *Acta clin. belg.* **10:** 369, 1955.
11. ALLEN, W. M.: A simple method for analyzing complicated absorption curves, of use in the colorimetric determination of urinary steroids, *J. Clin. Endocrinol.* **10:** 71, 1950.
12. BROWN, J. H. U.: An improvement of the Reddy method for the determination of 17-hydroxycorticoids in urine, *Metabolism* **4:** 295, 1955.
13. DE RAIMONDO, V. C.; ORR, R. H.; ISLAND, D.: RINFRET, A. P., and FORSHAM, P. H.: An improved steroidogenic assay of ACTH in man, *Metabolism* **4:** 110, 1955.
14. CRABBE, J., and HERTOGHE, J.: L'intérêt clinique du dosage des 17-hydroxycorticoïdes urinaires par la méthode de Reddy, *Acta clin. belg.* **10:** 397, 1955.
15. BORTH, R.; LINDER, A., and RIONDELL, A.: Urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids in healthy subjects in relation to sex, age, body weight and height, *Acta endocrinol.* **25:** 33, 1957.
16. NORYMBERSKI, J. K.; STUBBS, R. D., and WEST, H. F.: Assessment of adrenocortical activity by assay of 17-ketogenic steroids in urine, *Lancet* **1:** 1276, 1953.
17. LEVELL, M. J.; MITCHELL, F. L.; PAINE, C. G., and JORDAN, A.: The clinical value of urinary 17-ketogenic steroid determinations, *J. Clin. Path.* **10:** 72, 1957.
18. PRUNTY, F. T.: Chemical and clinical problems of the adrenal cortex, *Brit. M. J.* **2:** 615. 1956.
19. MARKS, L. J., and LEFTIN, J. H.: A note of caution on the lack of specificity of the Porter-Silber reaction for 17,21-dihydroxy-20-ketosteroids, *J. Clin. Endocrinol. & Metab.* **14:** 1263, 1954.
20. HUIS IN'T VELD, L. G.: Some critical remarks about the methods for the determination of steroid hormones in blood and urine, Proc. 3rd Internat. Congress Biochemistry, Brussels, Belgium, 1955, pp. 515–518.
21. GALLAGHER, T. F.; BRADLOW, H. L.; FUKUSHIMA, D. K.; BEER, C.; KRITCHEVSKY, T. H.; STOKEM, M.; EDINOFF, M. L.; HELLMAN, M. L., and DOBRINER, K.: Studies of the metabolites of isotopic steroid hormones in man, *in* Recent Progress in Hormone Research, ed. by G. Pincus. New York, Academic Press Inc., 1954, Vol. 9, pp. 411–433.
22. MIGEON, C. J.; SANDBERG, A. A.; DECKER, H. A.; SMITH, D. F.; PAUL, A. C., and SAMUELS, L. T.: Metabolism of 4-C^{14}-cortisol in man: body distribution and rates of conjugation, *J. Clin. Endocrinol. & Metab.* **16:** 1137, 1956.

Editorial

"METABOLIC INSUFFICIENCY"

SEVERAL recent papers and a vigorous advertising campaign by certain drug manufacturers have renewed the interest of clinicians in the problem of the patient who has a low basal metabolic rate without myxedema. The concept currently is advocated by some that the condition of such a patient constitutes a distinct clinical entity which is termed "metabolic insufficiency." "Metabolic insufficiency" is said to result from failure of peripheral tissues to de-iodinate thyroxine to the active, tissue form of thyroid hormone. Hence, a patient said to have this syndrome is refractory to treatment with desiccated thyroid substance or *l*-thyroxine, but will respond decisively to 3:5,3′-*l*-triiodothyronine.

The current interest in "metabolic insufficiency" was initiated by the report of Kurland, Hamolksy and Freedberg (1, 2), who studied 4 men with low basal metabolic rates. These patients suffered from lethargy, easy fatigability, nervousness, emotional instability, irritability, sensitiveness to cold, headaches, ill-defined skeletal pain and some reduction of sexual potency. These symptoms had proved refractory to treatment with desiccated thyroid substance. Despite basal metabolic rates of −20 to −25 per cent of the normal standard, these patients were considered to have normal thyroid function because the values for thyroidal uptake of radioiodine, serum protein-bound iodine and serum cholesterol were normal. In the first 3 patients, treatment with *l*-thyroxine, in dosages of 0.1 to 0.4 mg. per day, produced minimal metabolic response and no relief of symptoms. Triiodothyronine administered alone in moderate doses, or combined with *l*-thyroxine in small doses, rapidly elevated the basal metabolic rates and decisively relieved the symptoms. Subsequently, in 2 of the patients, the use of *l*-thyroxine alone maintained normal metabolic rates at dosage levels which previously had been ineffective, a result attributed to previous treatment with triiodothyronine.

Treatment of the fourth patient with 35 micrograms per day of triiodothyronine (a small dose), combined with 0.2 mg. per day of *l*-thyroxine, was followed by more marked elevation of the metabolic rate and more marked relief of symptoms than previously observed during treatment with 0.3 mg. daily of *l*-thyroxine alone.

Tittle (3) also reported 8 cases of "metabolic insufficiency," as defined

by Kurland and associates. These patients had basal metabolic rates between −28 and −36 per cent and normal thyroid function, as determined by appropriate tests. Six responded more decisively to triiodothyronine than to *l*-thyroxine, whereas 2 responded better to *l*-thyroxine used alone.

These observations have raised several important questions. Is "metabolic insufficiency" a distinct syndrome? If so, what are the criteria for recognition of it? How common is it? What physiologic mechanism is involved?

The symptoms of "metabolic insufficiency" would impress most physicians as being vague and nonspecific. "Lethargy, fatigability, nervousness, irritability, emotional instability, sensitivity to cold, headache and so on" obviously occur in a large proportion of patients, many of whom have anxiety tension states or psychoneurosis and some of whom have serious organic disease. As pointed out in a *Lancet* editorial (4) on this subject, one or more such symptoms can be anticipated in a majority of patients who consult physicians. Moreover, these symptoms *in the aggregate* are unlike those usually elicited from patients who have classic myxedema.

Patients who have myxedema may present relatively few complaints compared with the objective evidence of their metabolic disability. In fact, myxedematous patients often do not complain at all of symptoms referable to metabolic deficiency! Such symptoms as fatigability, nervousness, emotional instability, irritability or headaches in a patient who has frank myxedema frequently can be attributed to an associated psychoneurotic disturbance rather than to deficiency of thyroxine in itself. Furthermore, replacement therapy usually does not alleviate these complaints, even when the hypothyroid state is completely corrected.

Apart from symptoms considered by themselves, the diagnosis of "metabolic insufficiency" appears to hinge upon the association of the symptoms with hypometabolism. Interest in low basal metabolic rates in the absence of clinically discernible myxedema (also called "extrathyroidal hypometabolism") was intense about thirty years ago, after introduction of the clinical estimation of the basal metabolic rate. Current discussions of metabolic insufficiency generally fail to take full account of the extensive and careful observations then recorded. Metabolic rates lower than −15 per cent occurred in 1.2 per cent of the 8,614 patients studied by Boothby and Sandiford (5), and in 1.6 per cent of the first 1,000 patients studied by Means and Burgess (6). Numerous other writers have reported similar observations (7–14). Many of these patients were healthy; some had various menstrual irregularities (7); some, nasal conditions, such as rhinorrhea; and a substantial number (but by no means a majority) had a group of symptoms analogous to those described as characteristic of "metabolic

insufficiency." For example, among 196 patients with hypometabolism without myxedema whose records were carefully analyzed by Thurman and Thompson (15) in 1930, 11 were classified as healthy, 13 were mildly hypothyroid, 21 had ovarian dysfunction and 72 had miscellaneous conditions affecting the metabolic rate, such as undernutrition, anorexia nervosa, pituitary tumor or muscular atrophy. Seventy-nine presented a gamut of symptoms which would characterize the state now called "metabolic insufficiency."

In several recent papers it has been suggested that "metabolic insufficiency" may be common. Thus, Fields (16) reported 40 instances of "metabolic insufficiency" among 100 children treated with triiodothyronine. In the same paper were reported favorable responses to triiodothyronine in the treatment of 6 children with primary myxedema, 16 with pituitary myxedema and 38 with hypothyroidism associated with mental retardation, allergy, diabetes insipidus, achondroplasia, mongolism or cerebral palsy. The basis for these diagnoses is unclear, since the diagnostic criteria employed were not enumerated nor was laboratory evidence provided. The author advocated estimation of bone age as the ultimate criterion for the diagnosis of either hypothyroidism or "metabolic insufficiency" in children. However, he gave diagnostic weight to bone age "delayed" as little as 6 to 12 months, whereas most experienced radiologists and pediatricians would not consider such trivial variations as significant. The author suggested that a diagnosis of "metabolic insufficiency" may be based solely on the symptoms and the subjective response to treatment, without regard to the presence or absence of a low basal metabolic rate. Considering the prevalence and nonspecific character of the symptoms (and the suggestibility of many patients), such advice obviously invites this diagnosis in a sizable percentage of the "patient" population!

In another study concerned with the incidence of this condition, Morton (17) reported upon 51 patients with "metabolic insufficiency," 46 of whom were said to have been relieved of symptoms by triiodothyronine. In the absence of controls, such as placebos or "double-blind" technic, the significance of this report cannot be assessed. Morton's patients were characterized chiefly by chronic fatigue (88 per cent) and obesity (75 per cent); other symptoms or signs enumerated included menstrual disturbances, dry hair and skin and brittle fingernails, as well as irritability, depression, mental apathy, muscular aching and stiffness, sensitivity to cold and puffiness of the face. His patients were recorded as having normal values for serum protein-bound iodine or exhibiting normal reactions to tests with radioactive iodine, but they had significantly low basal metabolic rates (although basal metabolic rates as high as +2 per cent were included!).

In contrast to these 2 reports, Kurland and associates have been able

to find, over a period of several years' experience with this disease, only 15 patients with the condition as they define it (18). The relative paucity of reports on "metabolic insufficiency" from other centers perhaps might be interpreted as indicating that others also may have experienced difficulties in recognizing this condition.

Obviously, how often a diagnosis of "metabolic insufficiency" is made (provided the condition is looked for) also will depend upon the strictness with which criteria for recognition of the state are met. Since it is not feasible to measure basal metabolic rates in each of the many patients presenting one or more of the symptoms already listed, physicians are tempted to make an empirical trial of triiodothyronine as a diagnostic test. The original studies of Kurland and associates show how difficult it is to evaluate the results of such a therapeutic test. Through the years, the therapeutic trial as a means of diagnosis in clinical practice has proved to be misleading indeed. Familiar examples, which had only transient favor, were the therapeutic trials used in vitamin deficiencies (requiring vitamin preparations), iron deficiency (requiring iron) or pseudopernicious anemia (requiring liver). The treatment of fatigue with choline is another example. The practicing physician is, of course, often in a poor position from which to exercise critical judgment as to the relative efficacy of drugs in his patient. The patient consults a physician because he wishes to feel better, and the physician treats the patient with a sincere desire to oblige. Hence, the two together unconsciously conspire to produce a result pleasing to both.

The specific problem of evaluating the effects of a calorigenic drug in a patient with a low metabolic rate has additional inherent difficulties. It is difficult to compare desiccated thyroid substance or *l*-thyroxine, each of which works slowly, with triiodothyronine, which works less slowly. *Subjective* results of treatment with any of these agents may depend upon the period of observation and the rapidity with which the dose is increased, as well as upon qualitative differences in modes of action. Such well-known factors operative in subjective responsiveness have not been considered.

A therapeutic trial of triiodothyronine involves one pitfall which is unique and has not been sufficiently emphasized. During treatment with this agent, the values for serum protein-bound iodine *decrease*. This paradoxic effect may lead the physician unfamiliar with this phenomenon to believe even more firmly that his original impression was correct. Indeed, he may be led thereby to increase the dose of triiodothyronine to amounts which will produce hyperthyroidism!

A provocative aspect of "metabolic insufficiency" is the suggestion that it is a condition in which triiodothyronine plays a unique role by exerting an effect not obtainable with thyroxine. It is of extraordinary theoretic as well as clinical interest to determine whether or not this be true. Kurland

and associates suggested several possible mechanisms which might account for the action of triiodothyronine.

First, perhaps triiodothyronine is better absorbed than either desiccated thyroid substance or thyroxine. Many observers have noted that some patients with low basal metabolic rates require larger doses of desiccated thyroid substance to elevate their rates than do myxedematous patients who have comparable rates. Winkler and associates (19), however, observed by direct study that thyroid hormone is destroyed, inactivated or possibly excreted more rapidly in hypometabolic patients without myxedema than it is in normal persons. Furthermore, they found that thyroxine administered intravenously was even less effective in the hypometabolic patients, suggesting that impaired absorption probably was not responsible.

Second, perhaps patients with "metabolic insufficiency" have peripheral insensitivity to thyroxine. Kurland and associates suggested that thyroxine is not converted to an active form, or that the presence of triiodothyronine is necessary for the metabolism of thyroxine. This suggestion is of particular interest in light of the studies *in vitro* of Albright and others (20) showing that the de-iodination of thyroxine to tri-iodothyronine may occur in the kidney and the liver. The possibility, originally suggested by Gross and Pitt-Rivers (21), thus exists that triiodothyronine may be the peripheral form of thyroid hormone or a more immediate precursor of it.

Direct evidence is lacking that tissue hypometabolism exists in patients who have "metabolic insufficiency" and that a defect exists in the metabolism of thyroxine or the production of triiodothyronine. Such evidence obviously will be difficult to obtain. In a recent study, Kurland and associates (22) approached this problem by measuring the turnover of I^{131}-labeled thyroxine after intravenous infusion in 12 patients who had "metabolic insufficiency" and in 10 normal persons. Radiothyroxine disappeared from the blood at a distinctly lower rate in the hypometabolic group than it did in the normal persons. The mean half-value time was 8.3 days (range = 6.3 to 9.8 days) in the hypometabolic group, and 7.2 days (range = 6.6 to 7.8 days) in the group of normal persons. Other dimensions of thyroxine metabolism were similar in the 2 groups, but the retarded rate of disappearance was taken as evidence of defective thyroxine metabolism in the presence of "metabolic insufficiency."

This conclusion is difficult to accept when one considers other conditions that are associated with low basal metabolic rates (such as pituitary insufficiency and athyreosis with myxedema (23)) in which retarded rates for the disappearance of thyroxine have been demonstrated. In the presence of these conditions, there is no reason to impugn peripheral thyroxine metabolism as a cause of delayed disappearance of thyroxine. Furthermore,

not only radiothyroxine but also nonthyroidal material, such as radioiodine-labeled human serum albumin, may disappear from plasma more slowly in patients with a low basal metabolic rate and more rapidly in patients with a high rate (23, 24). Thus, the significance of the delayed rate of disappearance of thyroxine in the presence of "metabolic insufficiency" is not clear.

It would seem that physicians had best regard "metabolic insufficiency" at present as "unfinished business." Although there needs to be established the reality of a syndrome which can properly bear the label of "metabolic insufficiency," as defined, this must be done by demonstration beyond doubt that there are patients with similar groups of symptoms who do indeed respond to triiodothyronine but do not respond to thyroxine. The existence of the syndrome can be established only by means of clinical studies in which blind or double-blind technics are employed. In addition, fundamental medical investigations like those of Kurland, Hamolsky and Freedberg must be pursued with vigor if a rational explanation for the nature of the syndrome is to be given in terms of a unique physiologic defect. Until such evidence is forthcoming, physicians will be well advised to decide between well-reasoned experimental hypotheses and clinical observation on the one hand, and overenthusiastic and irresponsible advertising on the other.

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REFERENCES

1. Freedberg, A. S.; Kurland, G. S., and Hamolsky, M. W.: Effect of L-tri-iodothyronine alone and combined with L-thyroxine in nonmyxedematous hypometabolism: preliminary report, *New England J. Med.* **253**: 57 (July 14) 1955.
2. Kurland, G. S.; Hamolsky, M. W., and Freedberg, A. S.: Studies in non-myxedematous hypometabolism. I. The clinical syndrome and the effects of triiodothyronine, alone or combined with thyroxine, *J. Clin. Endocrinol. & Metab.* **15**: 1354 (Nov.) 1955.
3. Tittle, C. R.: Effects of 3,5,3′ L-triiodothyronine in patients with metabolic insufficiency, *J.A.M.A.* **162**: 271 (Sept. 22) 1956.
4. Editorial: Triiodothyronine for "metabolic insufficiency," *Lancet* **2**: 826 (Oct. 20) 1956.
5. Boothby, W. M., and Sandiford, I.: Summary of the basal metabolism data on 8,614 subjects with especial reference to the normal standards for the estimation of the basal metabolic rate, *J. Biol. Chem.* **54**: 783 (Dec.) 1922.
6. Means, J. H., and Burgess, H. W.: The basal metabolism in nontoxic goiter and in borderline thyroid cases: with particular reference to its bearing in differential diagnosis, *Arch. Int. Med.* **30**: 507 (Oct.) 1922.

7. HAINES, S. F., and MUSSEY, R. D.: Certain menstrual disturbances associated with low basal metabolic rates without myxedema, *J.A.M.A.* **105**: 557 (Aug. 24) 1935.
8. STURGIS, C. C.: A clinical study of myxedema with observations of the basal metabolism, *M. Clin. North America*, March, 1922, pp. 1251–1286.
9. LAWRENCE, C. H.: Thyroid failure without myxedema, *M. Clin. North America*, May, 1925, pp. 1779–1788.
10. WISHART, G. M.: Variability of basal metabolism, *Quart. J. Med.* **20**: 193 (Jan.) 1927.
11. LAWRENCE, C. H.: Physical background for symptoms of thyroid failure, with consideration of results of treatment, *Boston M. & S. J.* **196**: 43 (Jan. 13) 1927.
12. HIGGINS, W. H.: Management of hypothyroid patient, *South. M. J.* **20**: 779 (Oct.) 1927.
13. OHLER, W. R., and ULLIAN, L. J.: Clinical survey of 1000 cases on whom basal metabolism studies have been made, *M. Clin. North America*, March, 1925, pp. 1495–1509.
14. KIRK, E., and KVORNING, S. A.: Hypometabolism: a clinical study of 308 consecutive cases, *Acta med. scandinav.* Suppl. 184, pp. 7–83, 1946.
15. THURMON, F. M., and THOMPSON, W. O.: Low basal metabolism without myxedema, *Arch. Int. Med.* **46**: 879 (Nov.) 1930.
16. FIELDS, E. M.: Treatment of metabolic insufficiency and hypothyroidism with sodium liothyronine: preliminary report, *J.A.M.A.* **163**: 817 (Mar. 9) 1957.
17. MORTON, J. H.: Sodium liothyronine in metabolic insufficiency syndrome and associated disorders: preliminary report, *J.A.M.A.* **165**: 124 (Sept. 14) 1957.
18. FREEDBERG, A. S.: Personal communication to the author.
19. WINKLER, A. W.; LAVIETES, P. H.; ROBBINS, C. L., and MAN, E. B.: Tolerance to oral thyroid and reaction to intravenous thyroxine in subjects without myxedema, *J. Clin. Invest.* **22**: 535 (July) 1943.
20. ALBRIGHT, E. C.; LARSON, F. C., and TUST, R. H.: In vitro conversion of thyroxin to triiodothyronine by kidney slices, *Proc. Soc. Exper. Biol. & Med.* **86**: 137 (May) 1954.
21. GROSS, J., and PITT-RIVERS, R.: Physiological activity of 3:5:3′-L-triiodothyronine, *Lancet* **1**: 593 (Mar. 22) 1952.
22. KURLAND, G. S.; BUSTOS, J. G.; HAMOLSKY, M. W., and FREEDBERG, A. S.: Studies in nonmyxedematous hypometabolism. II. Turnover of I^{131}-labeled thyroxine after intravenous infusion, *J. Clin. Endocrinol. & Metab.* **17**: 1365 (Nov.) 1957.
23. STERLING, K. and CHODOS, R. B.: Radiothyroxine turnover studies in myxedema, thyrotoxicosis, and hypermetabolism without endocrine disease, *J. Clin. Invest.* **35**: 806 (July) 1956.
24. BAUMAN, A.; ROTHSCHILD, M. A.; YALOW, R. S., and BERSON, S. A.: Distribution and metabolism of I^{131} labeled human serum albumin in congestive heart failure with and without proteinuria, *J. Clin. Invest.* **34**: 1359 (Sept.) 1955.

Letters to the Editor

HALF-LIFE OF RADIOTHYROXINE IN NON-THYROIDAL HYPOMETABOLISM

To the Editor:

Recently several articles have appeared proclaiming the efficacy of triiothyronine in the treatment of non-thyroidal hypometabolism or "the metabolic insufficiency syndrome" (1–3). Since, reportedly, thyroxine was not of benefit to these patients the suggestion was made that a defect might exist in the conversion of thyroxine to a more active form of the hormone (1, 2). If this were the case, the biologic half-life of injected radiothyroxine might be prolonged.

We have observed several patients with complaints of chronic fatigue, intolerance of cold, constipation, and a variety of neurotic symptoms who also had low basal metabolic rates. All had had thyroid therapy without benefit some time in the past. In none were the physical findings sufficient to warrant a diagnosis of myxedema. All were moderately obese. None had received thyroid medication for six months prior to study.

The biologic half-life of radioiodinated (I^{131}) thyroxine was determined in 4 of these patients using the method of Sterling and Chodos (4). These and other pertinent data were as follows:

Subject, Age & sex	Ht. (ins.)	Wt. (lbs.)	BMR (%)	Serum cholesterol (mg./100 ml.)	Serum PBI (μg./100 ml.)	Thyroidal I^{131} uptake (%)	Radiothyroxine half-life (days)
F. S., 62 F	62	145	−19 to −25	430 to 600	5.7	26.7	5.9
M. R., 26 M	71	195	−23 to −28	168	6.0	33.0	5.7
R. H., 55 F	64	204	−18, −20	308	6.5	—	14.0
H. N., 23 F	63	145	−24, −22	300	8.0	—	8.7

The most likely reason for the low basal metabolic rates in these patients is a disproportion between the mass of metabolizing cells and the mass of fat. We do not feel that it is necessary to invoke any disorder of thyroid hormone metabolism. The normal data for Subjects F.S., M.R. and H.N.

lend support to this contention. Nevertheless, the half-life of radiothyroxine in Subject R.H. was longer than normal and is without explanation.

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REFERENCES

1. KURLAND, G. S.; HAMOLSKY, M. W., and FREEDBERG, A. S.: Studies in non-myxedematous hypometabolism. I. The clinical syndrome and the effects of triiodothyronine, alone or combined with thyroxine, *J. Clin. Endocrinol. & Metab.* **15:** 1354, 1955.
2. FREEDBERG, A. S.; KURLAND, G. S., and HAMOLSKY, M. W.: Effect of L-triiodothyronine alone and combined with L-thyroxine in non-myxedematous hypometabolism, *New England J. Med.* **253:** 57, 1955.
3. FIELDS, E. M.: Treatment of metabolic insufficiency and hypothyroidism with sodium liothyronine. Preliminary report, *J.A.M.A.* **163:** 817, 1957.
4. STERLING, K., and CHODOS, R. B.: Radiothyroxine turnover studies in myxedema, thyrotoxicosis, and hypermetabolism without endocrine disease, *J. Clin. Invest.* **35:** 806, 1956.

CONTRIBUTION TO THE STUDY OF ADRENOCORTICAL SECRETORY FUNCTION IN THYROTOXICOSIS

TO THE EDITOR:

Clinical observations and a study of the histopathologic findings in thyrotoxic patients suggest that their adrenals are insufficient compared with those of healthy subjects (1–5). On the other hand, the results of laboratory examinations indicate that in the majority of cases the secretory activity of the adrenal cortex is normal in this disorder (6–9). It is possible that long duration of the state of thyrotoxicosis results in marked depletion of the functional reserves of the adrenal cortex, but not to such an extent that insufficiency can be reflected in the values for adrenocortical steroids either in urine or plasma. In an effort to verify this hypothesis, we examined the actual and potential reserves of the adrenal cortex in cases of thyrotoxicosis by observing the levels of 17-hydroxycorticoids (17-OH-CS) after infusion of the adrenocorticotropic hormone (ACTH).

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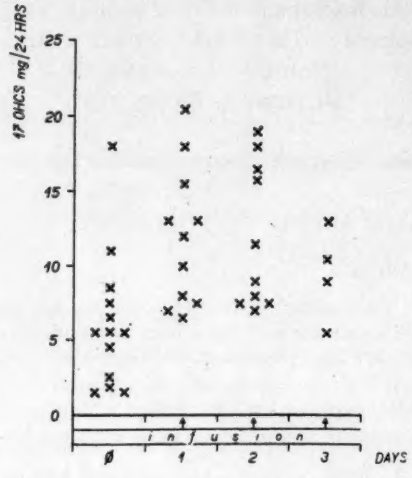

FIG. 1. Urinary 17-OH-CS excretion in thyrotoxic patients after infusions (arrows) of 25 units of ACTH.

In the first series of patients the values for the 24-hour urinary excretion of 17-OH-CS increased twofold after an initial 8-hour infusion of 25 units of ACTH, compared with values observed on the control day. However, this increase did not continue, in spite of repeated infusions of ACTH on the following days (Fig. 1). This is interpreted as a sign of the lowered potential reserve of the adrenal cortex in thyrotoxic patients. Nevertheless, since the significance of urinary 17-OH-CS values as an index of adrenal function may be uncertain in hypermetabolic states, we extended our investigation to include determination of the levels of 17-OH-CS in plasma (10).

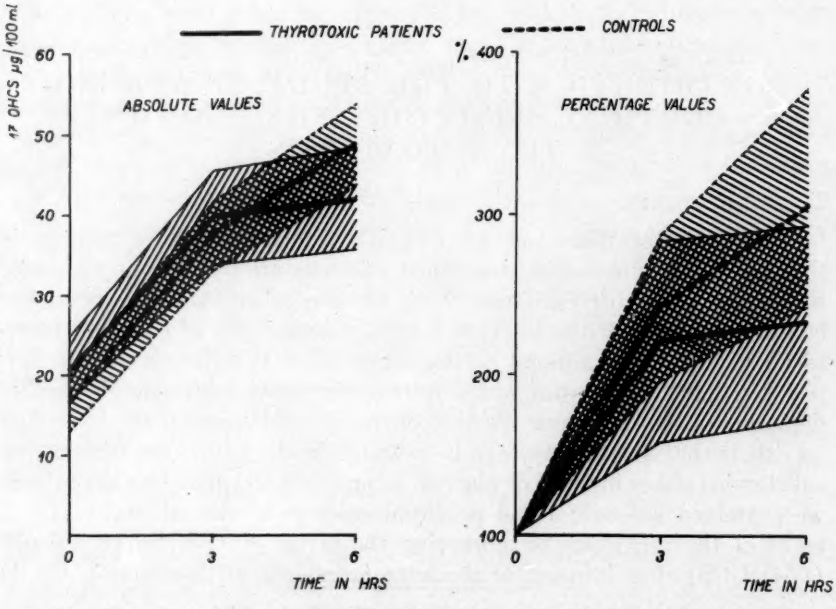

FIG. 2. Increase in plasma 17-OH-CS levels during a 6-hour infusion of 6.25 units of ACTH in thyrotoxic patients and in healthy controls.

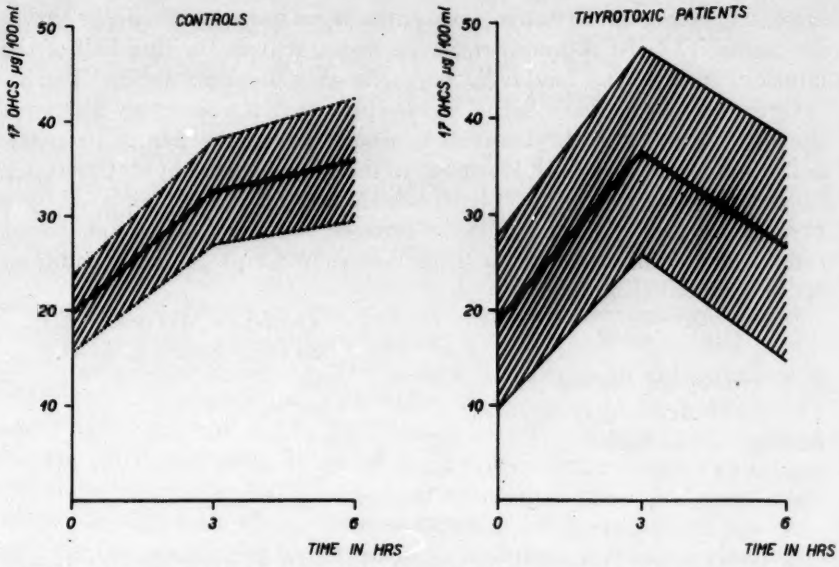

FIG. 3. Changes in plasma 17-OH-CS levels during a 6-hour infusion of 1 unit of ACTH in thyrotoxic patients and in healthy controls.

All patients in the study were suffering from severe, untreated thyrotoxicosis of long standing. In 68 observations, we found no significant difference between plasma 17-OH-CS values in 41 thyrotoxic patients and in 27 normal control subjects—in both, the mean level was 18 µg. per 100 ml.

Considering increased levels of plasma 17-OH-CS following administration of ACTH as a criterion of adrenocortical secretory function, we used the conventional ACTH-infusion test (25 units during six hours) in 11 thyrotoxic patients and in 19 healthy subjects. We failed to find any difference in plasma 17-OH-CS levels between the two groups.

We therefore endeavored to detect the suspected quantitative difference in secretion by using a lower dose of infused ACTH. In further studies we administered 6.25 units of ACTH intravenously in a six-hour infusion. With this dose, likewise, there was no significant difference in plasma 17-OH-CS levels between 13 thyrotoxic patients and 21 controls, though several of the curves in the thyrotoxic patients showed a decreasing trend in the second half of the infusion (Fig. 2).

The dose of infused ACTH was therefore reduced to 1 unit during six hours—representing submaximal stimulation of the adrenal cortex. With this dose, the curves of the plasma levels of 17-OH-CS were somewhat lowered in 16 healthy subjects, yet their trend was clearly of a steadily

increasing character. In 9 thyrotoxic patients, on the other hand, the curves for plasma 17-OH-CS concentration increased only in the first half of the infusion; during the second half, they invariably showed a decline (Fig. 3).

Our observations have led us to conclude that the secretory ability of the adrenal cortex in thrytoxicosis is usually adequate when under maximal stimulation by ACTH. However, in the course of *prolonged* stimulation by *submaximal* doses of ACTH, this adequate response is replaced by a phase of lowered secretion. In thyrotoxicosis, therefore, the adrenal cortex can not make full use of its potential reserve under physiologic conditions of submaximal stimulation.

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REFERENCES

1. SHAPIRO, S., and MARINE, D.: Clinical report of a case of Graves' disease with rapid improvement following the oral administration of fresh ox suprarenal gland, *Endocrinology* **5**: 699, 1921.
2. MARINE, D.: Remarks on pathogenesis of Graves' disease, *Am. J. M. Sc.* **180**: 767, 1930.
3. JÄRVINEN, K. A. J.: Beschädigungen der Nebennieren bei Morbus Basedowi; eine klinische Untersuchung, *Ann. med. int. Fenniae* **42**: 96, 1953.
4. HARRIS, G. W.: Hypothalamic regulation of anterior pituitary secretion, *Schweiz. med. Wchnschr.* **86**: 1252, 1956.
5. LECOMPTE, P. M.: Width of adrenal cortex in lymphatic leukemia, lymphosarcoma and hyperthyroidism, *J. Clin. Endocrinol.* **9**: 158, 1949.
6. BEIERWALTES, W. H., and BISHOP, R. C.: 17-Ketosteroid and pituitary follicle-stimulating hormone excretion in myxedema, before and during treatment with thyroxine, *J. Clin. Endocrinol. & Metab.* **14**: 928, 1954.
7. HERNBERG, C. A., and LAMBERG, B.-A.: Adrenal function and thyrotoxicosis, *Acta endocrinol.* **18**: 41, 1955.
8. LEVIN, M. E., and DAUGHADAY, W. H.: The influence of the thyroid on adrenocortical function, *J. Clin. Endocrinol. & Metab.* **15**: 1499, 1955.
9. SELENKOW, H. A.; SHEPPARD, R. W., and REDDY, W. J.: Adrenocortical function in hypothyroidism and hyperthyroidism, (abstract) *J. Clin. Endocrinol. & Metab.* **16**: 981, 1956.
10. SILBER, R. H., and PORTER, C. C.: Determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma, *J. Biol. Chem.* **210**: 923, 1954.

METHOD FOR DEMONSTRATING INCREASED INACTIVATION OF PITRESSIN IN DIABETES INSIPIDUS

To the Editor:

The lack of antidiuretic hormone (ADH) in diabetes insipidus may result not only from a decreased production of ADH in the hypothalamo-hypophyseal system but also from increased inactivation of ADH by the organism. We succeeded in verifying the latter possibility in some cases of diabetes insipidus (1) by a method in which Pitressin-inactivation could be determined *in vivo* in the human subject.

The method was as follows: The patient is loaded with water (at body temperature) through a duodenal tube. This serves 1) to restrain the patient's own production of ADH, and 2) to attain a high rate of urine flow. The urine is collected by catheter and is measured every five minutes. After these five-minute fractions have attained a constant level, a definite amount (50–200 mU) of Pitressin[1] is injected intravenously. The water loading is continued. The length of time taken for the rate of urine flow to reach its original level represents the Pitressin-inactivation ability of the organism.

By this method, increased inactivation of Pitressin was found in 4 patients with diabetes insipidus (2), in contrast with the results in simultaneously tested healthy controls.

We wish to report a modification of our method for demonstrating ADH inactivation, which yields better results. The major change consists of administering 2 doses of Pitressin instead of 1. The doses are given intravenously, half an hour apart. The period of depression of diuresis is thus considerably prolonged. The double administration avoids the use of a large initial dose which may cause distressing side-effects. A large dose may also induce counter-regulation, and thus cause misleading results.

Using this method, we found that the rate of urine flow became normal in a patient with diabetes insipidus one and a half hours after the first injection, but in a healthy control subject tested simultaneously the flow had not returned to the original level even after two hours. Thus, with this new modification of our *in vivo* Pitressin-inactivation test, a positive result was obtained in a patient with diabetes insipidus in whom the single-dose technique had yielded a normal result.

The double-administration method, therefore, may be valuable in the

[1] The author gratefully acknowledges generous supplies of Pitressin from Parke-Davis & Company.

detection of latent and mild disorders of ADH inactivation which may not be evident with the single-dose test.

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REFERENCES

1. HANKISS, J.: Ein Fall von Diabetes Insipidus, der Pitressin-refraktär schien, *Münch. med. Wchnschr.* **99**: 1418 (Sept.) 1957.
2. HANKISS, J.: Zum Problem der Geweblichen Pitressin-Inaktivierung bei Patienten mit Diabetes Insipidus, *Deut. Arch. klin. Med.* (in press) 1957.

The Endocrine Society

THE 1958 ANNUAL MEETING

The Fortieth Annual Meeting of The Endocrine Society will be held in the St. Francis Hotel, San Francisco, California, Thursday, Friday and Saturday, *June 19, 20, and 21, 1958.*

The Committee on Local Arrangements—Dr. Roberto Escamilla, Chairman; Dr. Donald E. Bernstein, Dr. Peter H. Forsham, Dr. Minnie B. Goldberg, Dr. Gilbert S. Gordan, Dr. Francis S. Greenspan, Dr. C. H. Li, as members of the committee.

All Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily in the Colonial Ballroom, and in addition there will be simultaneous afternoon sessions in the Italian Room. The annual dinner is scheduled for Friday, June 20 at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All reservations for rooms must be made through the AMA Housing Bureau, Room 300, 61 Grove Street, San Francisco 2, California. If possible, official application forms as published in the Journal of the American Medical Association should be used. Arrival and departure dates must be stated. All requests must be accompanied by check or money order (not cash) made payable to "The AMA Housing Bureau" in the amount of $10.00 per room, which will be credited to your hotel account. Be sure to state your first, second and third choice of hotels. It is important to make reservations immediately in order to avoid disappointment.

Final program, membership card and advance registration forms will be sent on May 15, 1958 to members whose current dues have been paid.

PROGRAM

THURSDAY, JUNE 19, 1958

8:30 A.M.—Registration—Mezzanine, St. Francis Hotel

Final session of the American Goiter Association program will be held in the Colonial Room. Those registered for The Endocrine Society meeting will be admitted to this meeting

I. 9:00 A.M.—ENDOCRINE AND METABOLIC INTERRELATIONSHIPS

Italian Room, St. Francis Hotel

Chairmen: *Leon Hellman and Dwight Ingle*

1. An Appraisal of the Extra-Adrenal Metabolic Actions of Corticotropin. *Frank L. Engel.*

2. Plasma Corticosterone Levels Following Transplantation of the Adrenal Gland to the Portal Circulation.
   Donald E. Bernstein, Gerson R. Biskind and *Nicolas Zenker* (by invitation).
3. Cortisol Suppression of Endotoxin Induced Serum Transaminase Elevation.
   James C. Melby, Richard H. Egdahl (by invitation) and *Wesley W. Spink.*
4. Some Observations Upon Adrenal Cortical Function During Pregnancy.
   Nicholas P. Christy, W. E. L. Gordon (by invitation), *Donald Longson* (by invitation), *Eleanor Z. Wallace* and *Joseph W. Jailer.*
5. Andrenocortical Hyperfunction in "Idiopathic" Hirsutism and the Stein-Leventhal Syndrome.
   T. F. Gallagher, Attallah Kappas, Leon Hellman, M. B. Lipsett, O. H. Pearson and *C. D. West.*
6. 17-Ketosteroid Fractionation in Virilism.
   Herman E. Carr, Jr. (by invitation), *William J. Reddy* and *George W. Thorn.*

INTERMISSION

7. Water Loading Test in Hypoadrenocorticism and Hypothyroidism.
   Arnold M. Moses (by invitation), *J. Lester Gabrilove* and *Louis J. Soffer.*
8. Body Composition and *in vitro* Synthesis of Lipids by Adipose Tissue of 11-Dehydrocorticosterone Treated Mice.
   Guy Hollifield and *William Parson.*
9. Absence of Direct Adrenal Cortical Effect Upon Lipogenesis by Rat Adipose Tissue.
   Bernard G. Jeanrenaud (by invitation) and *Albert E. Renold.*
10. An Adrenal Effect on the Production of Nephrotic Hyperlipemia.
    Peter R. Bally (by invitation) and *Luis Dominge* (by invitation), (introduced by Kendall Emerson, Jr.).
11. Blocking the Renal Electrolyte Effects of Mineralocorticoids with Synthetic Steroidal Lactones.
    C. J. Kagawa (by invitation), *J. A. Cella* (by invitation) and *C. G. Van Arman* (by invitation), (introduced by Francis J. Saunders).
12. Pharmacologic Inhibition of Steroid Oxygenation in the 11 Position in Man and Dog.
    John S. Jenkins (by invitation), *Lillian Pothier* (by invitation), *J. W. Meakin* (by invitation) and *Don H. Nelson.*

II. 2:00 P.M.—THYROID AND PARATHYROIDS

Colonial Room—St. Francis Hotel

Those who have registered with the American Goiter Association Meeting can attend this session without registering with The Endocrine Society meeting.

Chairman: *J. E. Rall and Sidney H. Ingbar*

13. The Failure of Acetyl-TSH or Pituitary Pepsin-Inhibitor to Alter the Thyroid Slice Response to TSH.
    John L. Bakke and *Nancy Lawrence.*
14. The Effect of Dinitrophenol on Thyroid Function in Rats with Hypothalamic Lesions.
    Seymour Reichlin.

15. The Effect of Hypophysectomy and of TSH on the Mouse Submaxillary Iodide Trap.
Alvin Taurog, G. D. Potter (by invitation) and *I. L. Chaikoff.*
16. Demonstration of Antithyroid Activity Following Ingestion of a Naturally-Occurring Thioglycoside.
J. M. Deeney (by invitation) and *Monte A. Greer.*
17. The Role of Thyroxine-Binding Interactions in the Thyroidal Release of Hormone.
J. Thomas Dowling (by invitation), *Sidney H. Ingbar* and *Norbert Freinkel.*
18. The Metabolism of S^{35}- and C^{14}-Labeled Thiourea by Thyroid Cell Particulate Fractions.
R. Maloof (introduced by J. B. Stanbury).

INTERMISSION

19. Steroid Hormone Metabolism in Myxedema.
H. Leon Bradlow, Leon Hellman, B. Zumoff and *T. F. Gallagher.*
20. Steroid Metabolism in the Thyroxin Treated Rat.
Joseph S. McGuire and *Gordon M. Tomkins* (introduced by J. E. Rall).
21. Alteration in Tubular Reabsorption of Phosphorus by Triiodothyronine.
Milton E. Rubini, William R. Beisel (by invitation), *Harold S. Ramos* (by invitation) and *William B. Blythe* (by invitation).
22. Comparison of Intravenous Strontium and Calcium Loads.
D. Werdegar, B. Clarkson and *M. Horwith* (introduced by Eugene J. Cohen).
23. Calcium Turnover in Man in Various Disease States.
E. Eisenberg (by invitation), *G. S. Gordan, R. Fraser* (by invitation) and *K. Ibbertson* (by invitation).
24. The Effects of Hypoparathyroidism, Acetazolamide, Parathormone, Dihydrotachysterol, and Vitamin D on Magnesium Metabolism in Man.
Leonard P. Eliel, William O. Smith (by invitation), *Carl Thomsen* (by invitation) and *Josephine Hawrylko* (by invitation).

III. 2:00 P.M.—ADRENAL AND GONADS

Italian Room, St. Francis Hotel

Chairmen: *J. W. Jailer and C. D. West*

25. Inhibitors of Adrenal Cortical Secretion.
Robert Gaunt, J. J. Chart, Nancy Howie (by invitation), *Betty Sullivan* (by invitation) and *H. Sheppard* (by invitation).
26. The Determination of Non-Protein Bound Plasma Hydrocortisone by Ultrafiltration.
Ivor H. Mills (by invitation), *Harold P. Schedl* (by invitation), *Diana Redd* (by invitation) and *Frederic C. Bartter.*
27. Metabolism of 4-C^{14}-Progesterone by Human Adrenal Microsomes.
W. R. Slaunwhite, Jr. and *A. A. Sandberg.*
28. Distribution of 16-C^{14}-Estrone Between Protein Fractions of Plasma and Red Blood Cells of Man.
Claude J. Migeon, Ono L. Lescure (by invitation) and *Harry N. Antoniades* (by invitation).

29. Identification of A Pregnancy Plasma Adrenotropin as Chorionic Gonadotropin.
Jonathan T. Lanman and *Jenny Dinerstein* (by invitation).
30. Treatment of Ovarian Dysfunction with Small Doses of Cortisone or Hydrocortisone.
W. McK. Jefferies and *R. P. Levy.*

INTERMISSION

31. Progestational Activity of Certain 19-Nor Steroids and a Progesterone Derivative.
Tamotsu Miyake.
32. 17-Ketosteroids of Meconium.
Nai-Hsuan Chang Shen, Faith Ellen Francis and *Ralph A. Kinsella Jr.* (introduced by Edward A. Doisy).
33. Effect of Norethandrolone on 17-Ketosteroid Excretion in Prostatic Cancer Patients.
Herbert Brendler and *Bertram S. Winkler* (introduced by William Money).
34. Inherent Estrogenicity of the Orally Active Progestational Steroids, Norethisterone and Norethynodrel.
C. Alvin Paulsen, Robert B. Leach, Norman Goldston (by invitation), *John Lanman* (by invitation) and William O. Maddock.
35. Effect of *in vivo* Administration of Estrogens on Rat Adrenal Corticoid Secretion *in vitro.*
Kenneth W. McKerns and *E. Kaleita* (by invitation).
36. Coexistence of Adrenal Virilizing Hyperplasia and Interstitial Cell Tumors in an Adult Male.
Stanley Newman, Gerold H. Mahaffey (by invitation) and *Vincent C. Di Raimondo.*

FRIDAY, JUNE 20, 1958

8:30 A.M.—Registration—Mezzanine, St. Francis Hotel

IV. 9:00 A.M.—PITUITARY AND HYPOTHALAMUS

Colonial Room, St. Francis Hotel

Chairmen: *A. E. Wilhelmi and Leslie L. Bennett*

37. A Simplified Method for the Isolation of Alpha and Beta Melanocyte Stimulating Hormones.
Sanford L. Steelman.
38. The Measurement of Serum Sulfation Factor as an Idea of Somatotrophin Activity in Man.
William H. Daughaday, William D. Salmon (by invitation) and *France Alexander* (by invitation).
39. Assay of Growth Hormone for Activity in Man.
M. S. Raben and *C. H. Hollenberg* (by invitation).
40. Human Growth Hormone: Prolonged Administration of Moderate Doses in Three Patients with Panhypopituitarism.
Philip H. Henneman, Marc Moldawer, Evelyn L. Carroll (by invitation), *Mary Mitchell* (by invitation) and *Mary Josephine Waddell* (by invitation).

41. Anabolic Effects of Bovine Growth Hormone in Man.
Ivar Werner (by invitation), and *Frederic C. Bartter.*
42. Evidence for Stimulation of Insulin Secretion by Growth Hormone (STH) in the Rat.
Frank L. Engel, Thomas Albertson (by invitation), *John Fredericks* and *Enrique Lopez.*

INTERMISSION

43. Differential Release of ACTH *in vivo* by a Hypophysiotropic Fraction and Purified Lysine Vasopressin.
Rober Guillemin, G. W. Clayton, J. D. Smith (by invitation), *H. S. Lipscomb* (by invitation), *D. N. Ward* (by invitation) and *A. V. Schally* (by invitation).
44. Mechanism of Inhibition of ACTH Release by Hydrocortisone.
A. Brodish and *C. N. H. Long.*
45. Effects of ACTH Upon Pituitary Responsiveness to Stress After Steroid Therapy in Rats.
Julian K. Kitay, Donald A. Holub (by invitation) and *Joseph W. Jailer.*
46. Some Effects of Hormone on Electrical Activity and Thresholds in the Brain.
M. Kawakami (by invitation) and *Charles H. Sawyer.*
47. Precocious Estrus in Rats with Hypothalamic Lesions.
E. M. Bogdanove and *H. Ç. Schoen* (by invitation).
48. Gonadotrophic Activity of the Periodic Acid-Schiff Reactive Material of Anterior Pituitary "Basophils" of Normal Rats.
Griff T. Ross (by invitation), *Robert C. Bahn* and *Robert W. Schmit* (by invitation).

1:30 P.M.—ANNUAL BUSINESS MEETING OF THE ENDOCRINE SOCIETY

V. 2:30 P.M.—GONADS AND REPRODUCTION

Italian Room, St. Francis Hotel

Chairmen: *Ralph I. Dorfman and Herbert Kupperman*

49. Urinary Gonadotropin Excretion Levels Measured by the Mouse Uterine Response Employing Estrone as the Standard Reference Material.
Eugenie Rosemberg.
50. A New Assay for Gonadotropins.
Warner H. Florsheim, Shirley M. Velcoff (by invitation) and *Ralph E. Bodfish* (by invitation).
51. Luteinizing Hormone and Ovulation.
Sheldon J. Segal.
52. Follicle Stimulation and Luteinization in Hypophysectomized Rhesus Monkeys.
E. Knobil, J. L. Kostyo (by invitation) and *R. O. Greep.*
53. Effect of Folic Acid and Glutamine Antagonists on Chorionic Gonadotropin Producing Tumors.
M. C. Li and *G. B. Magill* (by invitation).
54. Effect of Triethylenemelamine on Reproduction.
Emil Steinberger (by invitation), *W. O. Nelson* and *A. Boccabella* (by invitation).

INTERMISSION

55. Steroidal Hydroxylases of the Human Adrenal Gland.
Alfred M. Bongiovanni and *Walter R. Eberlein.*
56. α-Ketolic Estrogen (16α-Hydroxyesterone) Titers in Human Pregnancy Urine.
Benjamin F. Stimmel.
57. Steroid Ring A Aromatization by Human Placental Microsomes.
Kenneth J. Ryan (introduced by Lewis L. Engel).
58. The Effect of Fsh on the Conversion of Testosterone-C-14 to Estradiol-C-14 by Dog Ovarian Slices.
Nina Hollander (by invitation) and *Vincent P. Hollander.*
59. Pharmacologic and Clinical Appraisal of the Newer Progestational Steroids.
Jeanne A. Epstein, Jean Seidl (by invitation), *Arthur Cutler* (by invitation) and *Herbert S. Kupperman.*
60. Masculinization of the Female Fetus Associated with Administration of Oral and Intramuscular Progestins During Gestation.
Lawson Wilkins, Howard W. Jones, Jr. (by invitation), *Gerald H. Holman* (by invitation) and *Robert S. Stempfel, Jr.* (by invitation).

VI. 2:30 P.M.—DIABETES AND METABOLISM

Colonial Room, St. Francis Hotel

Chairmen: *F. D. Lukens and Frank Engel*

Those registered for the meeting of The American Diabetes Association will be admitted to this meeting.

61. The Effect of Insulin on Serum Lipoprotein Concentrations in Diabetic Patients.
Edward Strisower (introduced by F. D. W. Lukens).
62. Comparison of the Effects of Starvation and Alloxan Diabetes on Palmitic Acid Oxidation by Muscle.
Irving B. Fritz and *Harold Dundee* (by invitation).
63. The Effect of Various Steroids on the Hyperglycemic Action of Glucagon.
Shirley Weisenfeld and *Martin G. Goldner.*
64. Mechanism of the Glucosuria Produced by Repeated Injections of Glucagon in Normal Subjects.
Jean-Pierre Felbar and *Theodore B. Van Itallie* (introduced by F. D. W. Lukens)
65. Interrelationship Between Glucagon and Compound F in Protein Metabolism of Adrenalectomized Fasting Rats.
Stanley R. Glasser and *Joseph L. Izzo* (by invitation).
66. The Effect of Insulin on Hepatic Glucose Production in Normal Dogs.
Robert Mahler, William C. Shoemaker, and *Daniel E. Pugh* (introduced by James Ashmore).

INTERMISSION

67. Turnover and Oxidation of Plasma Glucose in Humans as Influenced by Insulin and Orinase.

G. L. Searle, G. E. Mortimore, R. Buckley and *W. A. Reilly* (introduced by F. D. W. Lukens).

68. THE EFFECTS OF 6-DEOXY-6-FLUOROGLUCOSE ON THE METABOLISM OF GLUCOSE.
Arne N. Wick (by invitation), *George S. Serif* (by invitation), *Ellen Larson* (by invitation) and *Charles J. Stewart* (by invitation).

69. EFFECTS OF 2-DEOXYGLUCOSE ON GLUCOSE METABOLISM IN THE RAT AND ON THE HYPOGLYCEMIC RESPONSE TO TOLBUTAMIDE (ORINASE).
Josiah Brown and *Howard L. Bachrach* (by invitation).

70. PATTERN OF CARBOHYDRATE AND FAT METABOLISM DURING PROLONGED, EFFECTIVE DOSES OF HUMAN GROWTH HORMONE IN MAN.
Dorothy H. Henneman (introduced by Maurice Pechet).

71. THE INHIBITION OF INSULIN DEGRADATION BY PLASMA FRACTIONS OF NON-DIABETIC SERA.
Charles J. Goodner (byinvitation), *Sidney H. Ingbar,* and *Norbert Freinkel.*

72. ENDOGENOUS INSULIN SECRETORY RESPONSE TO HYPERGLYCEMIA AS EVIDENCED BY ACUTE CHANGES IN PLASMA GLUCOSE-C-^{14}-CONCENTRATION.
S. A. Berson, S. Weisenfeld and *M. Pascullo* (by invitation).

ANNUAL DINNER—St. Francis Hotel

Leo Samuels, President, presiding.

6:30 P.M. Cocktails, sponsored by the donors of the Awards of the Society: Ciba, Schering, Ayerst, Upjohn and Squibb. Attendance limited to holders of dinner tickets.

7:30 P.M. Dinner

Presentation of 1958 Awards of the Society by *E. B. Astwood:*
The Medal of the Endocrine Society
Ciba Award
Squibb Fellowship
Schering Scholar
Upjohn Scholar

President's Address: *Dr. Samuels*

SATURDAY, JUNE 21, 1958

8:30 A.M.—Registration—Mezzanine, St. Francis Hotel

9:00 A.M. Opening scientific section of The American Diabetes Association will be held at the Mark Hopkins Hotel. Endocrine Society badge will admit you to this meeting.

VII. 9:00 A.M.—ADRENAL

Colonial Room, St. Francis Hotel

Chairmen: *John A. Leutscher, Jr. and Alfred M. Bongiovanni*

73. ADRENAL ASCORBIC ACID DEPLETION AND CORTICOSTEROID RELEASE IN HYPOPHYSECTOMIZED RATS WITH LOW DOSES OF ACTH.
Margaret A. Slusher (by invitation) and *Sidney Roberts.*

74. Potentiation of ACTH by Corticosteroid Precursors.
Irene T. Kline and *Richmond W. Smith, Jr.*
75. Pituitary ACTH and Plasma Free Corticosteroids During Regeneration of the Enucleated Rat Adrenal Gland.
Claude Fortier and *Jack De Groot* (by invitation).
76. Studies on the Functional Analysis of Hyperactive Adrenal States.
Vincent C. Di Raimondo, Dace B. Mitchell (by invitation), *Shirley Cook* (by invitation) and *Peter H. Forsham.*
77. Effect of Various Pituitary and Hypothalamic Extracts on Aldosterone Secretion.
E. H. Venning, O. J. Lucis (by invitation), *I. Dyrenfurth* (by invitation) and *J. C. Beck.*
78. Effects of Stress, Corticotrophin, and Steroids on the Adrenal Necrosis of Besnoitia-Infected Hamsters.
J. K. Frenkel (introduced by F. R. Skelton).

INTERMISSION

79. Chronic Adrenocortical Dysfunction, Including Aldosterone Deficiency: Studies of Steroid and Electrolyte Metabolism.
George D. Molnar (by invitation), *Vernon R. Mattox* (by invitation), *Harold L. Mason* and *Marschelle H. Power* (by invitation).
80. Aldosterone Excretion in Patients with Virilizing Adrenal Hyperplasia with Normal and Low Salt Intake.
Robert M. Blizzard (by invitation), *Grant W. Liddle* and *Claude J. Migeon.*
81. Aldosterone Secretion in Dogs with Hypothalamic Lesions.
W. F. Ganong, A. H. Liberman (by invitation), *P. J. Mulrow* (by invitation) and *J. A. Luetscher, Jr.*
82. The Metabolism of Tritiated Aldosterone in Man.
Bernard Kliman and *Ralph E. Peterson* (introduced by G. Donald Whedon).
83. Chronic Aldoesterone Administration in Normal Subjects.
J. Thomas August (by invitation), *Don H. Nelson* and *George W. Thorn.*
84. Primary Hyperaldosteronism Due to Hyperfunction of the Adrenal Cortex Corrected by Partial Adrenalectomy.
B. J. Kennedy, James Melby, Frederick Goetz, Walter Moran (by invitation) and *Bernard Zimmerman.*

SATURDAY, JUNE 21, 1958

CLINICAL PANELS

VIII. 2:00 P.M.—Endocrine Treatment of Neoplastic Disease.

Colonial Room, St. Francis Hotel

Chairman: *O. H. Pearson*

William H. Baker
Albert Segaloff
Herbert Brendler
B. J. Kennedy
Delbert Bergenstal

IX. 2:00 P.M.—HYPERPARATHYROIDISM AND BONE DISEASE.

Italian Room, St. Francis Hotel

Chairman: *Gilbert S. Gordan*

John Eager Howard
Laurence H. Kyle
Philip Henneman
Oliver Cope
W. P. Laird Myers

X. 3:30 P.M.—CHEMICAL TREATMENT OF DIABETES.

Colonial Room

Those registered for the meeting of The American Diabetes Association will be admitted to this meeting.

Chairman: *Peter Forsham*

Alexander Marble
Max Miller
Albert E. Renold
Henry Dolger
Robert Williams

XI. 3:30 P.M.—INTERSEXUALITY

Italian Room, St. Francis Hotel

Chairman: *Warren Nelson*

Melvin Grumbach
R. Hoffenberg
Herbert Kupperman
Robert Greenblatt

TO BE READ BY TITLE

(Abstracts* 85–188.)

*** Abstracts of Papers Appearing on the Program and Those Read by Title Will Not be Printed in the Journals This Year but Will be Printed in Full in the Annual Program.**

THE 1958 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955, Dr. Frederick L. Hisaw in 1956, and Dr. Joseph C. Aub in 1957.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.

3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1958 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the St. Francis Hotel, San Francisco, California, on *June 16, 17, 18 and 19, 1958.*

All reservations for rooms (accompanied by a $10 deposit) must be made through the AMA Housing Bureau, Room 300, 61 Grove Street, San Francisco 2, California.

PRELIMINARY PROGRAM

ST. FRANCIS HOTEL

June 16, 17, 18 and 19, 1958

Monday, June 16, 1958, 8:00 A.M., Moffitt Hospital, San Francisco: Operative Clinics, Panel Discussion—Carcinoma of the Thyroid Gland—and Afternoon Clinics. Admission by ticket only, for which please write Dr. Robertson Ward, 384 Post Street, San Francisco, California.

Program for wives of members and guests, Tuesday and Wednesday, June 17 and 18. Please purchase tickets at the Registration Desk.

Tuesday, June 17, 1958—8:30 A.M.

Observations Concerning the Heterogeneity of Human and Sheep Thyroglobulin.
Sidney H. Ingbar, Bridgitte A. Askonas and *Thomas S. Work,* Boston, Mass.

Non-Thyroglobulin Protein Iodine in Thyroid Tissue.
Jacob Robbins, J. Wolff and *J. E. Rall,* Bethesda, Md.

Surgical Management of Substernal Goiter.
Oliver H. Beahrs, Edward S. Judd and *Donald E. Bowes,* Rochester, Minn.

The Prevention and Treatment of Recurrent Nontoxic Nodular Goiter Using Thyroid Therapy.
Oliver Cope, Benjamin Barnes and *Rafael Casas,* Boston, Mass.

The Effect of Administration of Desiccated Thyroid to Patients with Hashimoto's Thyroiditis and Adenomatous Goiter without Hyperthyroidism.
William M. McConahey, Lewis B. Woolner, B. Marden Black and *F. Raymond Keating, Jr.,* Rochester, Minn.

A Comparison of the Biological Actions of Thyroxine and Its Analogues: Antigoitrogenically and Calorigenically in Rats.
N. R. Stasilli, R. L. Kroc and *R. I. Meltzer,* Morris Plains, N. J.

A Comparison of the Biological Actions of Thyroxine and Its Analogues: Tadpole Metamorphosis and Suppression of Rat Thyroid's Avidity for I^{131}.
William L. Money, R. I. Meltzer, Diane Feldman and *Rulon W. Rawson,* New York, N.Y.

Exophthalmic Goiter in Children.

Alvin B. Hayles, Roger L. J. Kennedy, Oliver H. Beahrs and *Lewis B. Woolner,* Rochester, Minn.

The Effect of Maternal Thyroid Function on Fetal Thyroid Function and Development.

E. A. Carr, Jr., W. H. Beierwaltes, G. Raman and *N. R. Spafford,* Ann Arbor, Mich.

Tuesday, June 17—2:00 P.M.

Tissue Metabolism of Thyroxine, Triiodothyronine, and Their Analogues.

B. Yamazaki and *D. W. Slingerland,* Boston, Mass.

The Metabolism of I^{131}-Labeled Thyroxine, Triiodothyronine, and Diiodotyrosine by Isolated Perfused Rabbit Liver.

David V. Becker and *John F. Prudden,* New York, N. Y.

The Effect of Estrogen in Peripheral Thyroxine Degradation.

J. Thomas Dowling, Norbert Freinkel and *Sidney H. Ingbar,* Boston, Mass.

Functional Relation Between the Liver, the Thyroxine-Binding Protein, and the Thyroid.

A. Vannotti, Lausanne, Switzerland.

Clinical Observations of Serum Globulin Thyroxine-Binding Capacity Using a Simplified Technique.

Paul Starr and *Shigeru Tanaka,* Los Angeles, Calif.

Butanol-Insoluble Serum Radioiodine in Hashimoto's Disease and in Cancer of the Thyroid.

Charles A. Owen, Jr., William M. McConahey and *F. Raymond Keating, Jr.,* Rochester, Minn.

Struma Lymphomatosa and Related Lymphocytic Alterations in the Thyroid Gland: A Clinicopathologic Study of Proved Cases.

Lewis B. Woolner, William McConahey and *Oliver H. Beahrs,* Rochester, Minn.

The Metabolic and Clinical Effects of 3:5:3′-Triiodothyronine in Patients with Various Psychopathological Conditions.

Frederick F. Flach, Charles I. Celian, Peter E. Stokes and *Rulon W. Rawson,* New York, N. Y.

Wednesday, June 18—8:30 A.M.

Hypophyseal Thyrotropin Content in Humans: A Preliminary Report.

John L. Bakke and *Nancy Lawrence,* Seattle, Wash.

Studies on the Effect of TSH on Human Thyroid Function.

Jerzy Einhorn and *L.-G. Larsson,* Stockholm, Sweden.

Problems in the Bioassay of Pituitary Thyrotropic Hormone in Blood and Urine.

Francis S. Greenspan and *William Lew,* San Francisco, Calif.

Cytochemical Changes in the Nucleus of Thyroids Radiated with I^{131}; A Measure of Desoxyribosenucleic Acid by Spectrophotometry.

Brown M. Dobyns and *Irene Didtschenko,* Cleveland, Ohio.

The Nucleic Acid Content of the Thyroid Gland.

Josip Matovinovic, Austin Vickery and *Harold Carter,* Boston, Mass.

Van Meter Prize Paper

Iodide Trapping Without Organification in a Rat Thyroid Carcinoma.

J. Wolff, Jacob Robbins and *J. E. Rall,* Bethesda, Md.

The Diagnostic and Therapeutic Value of Thyrotropic Hormone and Heavy Dosage Scintigrams for the Demonstration of Thyroid Cancer Metastases.

B. Catz, D. Petit and *P. Starr,* Los Angeles, Calif.

Occurrence of Thyroid Nodules in Children Following Radioiodine Therapy for Hyperthyroidism.

Glenn E. Sheline, Stuart Lindsay and *H. Glenn Bell*, San Francisco, Calif.

The Increasing Incidence of Carcinoma of the Thyroid in a Surgical Practice.

Melvin A. Block, J. Martin Miller and *Robert C. Horn*, Detroit, Mich.

Wednesday, June 18—2:00 P.M.

Presidential Address:

Elmer C. Bartels, Boston, Mass.

Medullary Carcinoma of the Thyroid: A Clinico-Pathologic Entity.

J. B. Hazard, W. A. Hawk and *George Crile, Jr.*, Cleveland, Ohio.

Parenchymal Findings in Thyroidal Carcinoma: Pathologic Study of 256 Cases.

Donald W. Meier, Lewis B. Woolner, William M. McConahey and *Oliver H. Beahrs*, Rochester, Minn.

Concretions (Psammoma Bodies, Calcospherites, or Microliths) and Thyroid Cancer: An Inquiry Into the Diagnostic Significance of Their Association.

G. H. Klinck and *T. Winship*, Washington, D. C.

Preoperative Diagnosis of Thyroid Carcinoma With Radioactive Phosphorus and Radioactive Iodine.

F. K. Bauer, B. Catz and *P. Starr*, Los Angeles, Calif.

Pulmonary Function in Patients with Hyperthyroidism.

Myron Stein, Philip Kimbel and *R. L. Johnson, Jr.*, Boston, Mass.

Thyroid Function in Supraventricular Tachycardias.

George S. Kurland, Arnold Golodetz, Milton W. Hamolsky and *A. Stone Freedberg*, Boston, Mass.

Thursday, June 19—8:30 A.M.

Further Studies on the *In Vitro* Incorporation of I^{131} Triiodothyronine by Human Erythrocytes as a Diagnostic Test of Thyroid Function.

Milton W. Hamolsky and *A. Stone Freedberg*, Boston, Mass.

Effect of Hypothalamic "Goiter Block" on TSH Synthesis in the Guinea Pig.

E. M. Bogdanove and *S. A. D'Angelo*, Albany, N. Y.

The Effect of Iodide on the Action of Thyrotropin in the Hypophysectomized Patient.

Richard S. Benua and *M. B. Lipsett*, New York, N. Y.

Hypothalamic-Adrenal-Thyroid Relationships.

G. W. Harris, London, England.

The Uptake of P^{32} as a Measure of Cellular Metabolism in the Human Thyroid.

Colin G. Thomas, Jr., Chapel Hill, N. C.

Comparison of Thyroidal and Gastric Iodide Pumps in Rats.

R. G. Stuelke and *N. S. Halmi*, Iowa City, Iowa.

The Effect of *l*-Thyroxine and Desiccated Thyroid on Serum Lipoproteins and Cholesterol.

Beverly Strisower, Paul Elmlinger, John W. Gofman and *Oliver de Lalla*, Berkeley, Calif.

The Behavior of Iodide in Acid Solutions of Oxyhemoglobin.

I. N. Rosenberg, Boston, Mass.

A Comparison of Adrenal Cortical Function in Hyper- and Hypothyroidism.

Vincent DiRaimondo, Leonard Sagan, Phillip Perloff, Satoshi Hane and *Peter H. Forsham*, San Francisco, Calif.